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CONTENTS

PAGE

BEAN, J., BRIAN, P. W., and BROOKS, F. T. Physiologic Races of Brown Rust of Brome Grasses 129

GODWARD, M. B. E. The 'Diffuse' Centromere or Polycentric Chromosomes in *Spirogyra*. With Plates V and VI and four Figures in the Text 143

MER, C. L. The Use of the Infra-red 'Image Converter' Viewing Apparatus in the Study of the Effects of Localized Wounds on the Growth of Etiolated *Avena* Seedlings. With Plate VII and three Figures in the Text 157

CUSICK, F. Experimental and Analytical Studies of Pteridophytes. XXV. Morphogenesis in *Selaginella Willdenovii* Baker. With Plate VIII and seven Figures in the Text . . 171

DREW, KATHLEEN M. Studies in the Bangioideae. III. The Life-history of *Porphyra umbilicalis* (L.) Kutz. var. *laciniata* (Lightf.) J. Ag. With Plates IX-XII and five Figures in the Text 183

HOPKINS, B. A New Method for determining the Type of Distribution of Plant Individuals. With one Figure in the Text 213

MAMAY, S. H. A New Sphenopsid Cone from Iowa. With Plates XIII-XIV and two Figures in the Text 229

HYDE, E. O. C. The Function of the Hilum in Some Papilionaceae in Relation to the Ripening of the Seed and the Permeability of the Testa. With nine Figures in the Text. . 241

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Physiologic Races of the Brown Rust of Brome Grasses[1]

BY

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AND

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ABSTRACT

A number of cultures of the brown rust of Brome grasses have been isolated and maintained as single-spore strains. By their infection reactions on a representative selection of *Bromus* spp. at least five physiologic races have been characterized. Two of these have host-ranges confined to a single section of the genus, but others have a much wider host-range within the genus. The uredospore stage of all was identical. Studies of the teleutospores have been limited and knowledge of the species of rust involved is incomplete. All belong to the group-species *Puccinia dispersa* Erikss. & Henn.; the species *P. bromina* Erikss. and *P. symphyti-bromorum* Müller were included among the cultures studied, possibly also *P. bromi-maximi* Guyot. In a large series of inoculations, over a period of in all 14 years, all physiologic races have been remarkably stable in their infection types and host-range, and no confirmation of Marshall Ward's 'bridging-host' theory has been obtained. Cultivation of two physiologic races for 164 generations on *Bromus arduennensis*, a species considered by Ward to have special significance as a bridging-host, induced no changes in their host-range.

INTRODUCTION

FIFTY years ago Marshall Ward (1902, 1903) and Freeman (1902) published a series of papers on the host-parasite relations of species of *Bromus* and a brown rust, *Puccinia dispersa* Erikss. & Henn. (nowadays usually referred to in this country as *P. bromina* Erikss.). This work has since become classical in that on the basis of the results obtained Ward developed his well-known 'bridging-host' theory. At that time the concept of physiologic races of such pathogens as the rusts was not as clear and well established as it has since become. Ward, however, was able to show that there were, to use his terminology, two or more 'adapted races' of the rust, each more or

---

[1] The experimental work described in this paper was begun by one of us (P. W. B.) when a research student at the Botany School, Cambridge. It was later continued by the other two authors. The project was initiated and supervised throughout by the late Professor F. T. Brooks, and two of us (J. B., and P. W. B.) wish here to record our appreciation of his interest and advice. This paper was written after Professor Brooks's death, but owes much to his very detailed experimental records.

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[Annals of Botany, N.S. Vol. XVIII, No. 70, April, 1954.]

less restricted to a group of closely allied species of the genus *Bromus*. He also showed that certain species might act as hosts to more than one race of the rust. He suggested, on the basis of such observations, that such common hosts, which he called 'bridging-species', could so change an adapted race that it could, after growth on the bridging-species, then pass to host species previously immune. In other words, he regarded an adapted race as an essentially plastic entity, whose parasitic potentialities could be decisively moulded by the host plant on which it was growing.

Such a concept is very much at variance with the subsequent 50 years' experience of research on physiologic races of cereal rusts. Stakman, Piemeisel, and Levine (1918) showed that physiologic races of *Puccinia graminis* were highly stable and they could find no evidence in support of Ward's views. Since then other workers have had similar experiences with other cereal rusts and the bridging-host theory is now generally rejected.

As Sampson and Western (1941) have pointed out, no further work on Brome rusts has been published in this country since Ward's time, and the recent French and Swiss work has been largely concerned with morphology in relation to taxonomy and nomenclature. A reinvestigation of the biology of these rusts, in the light of modern knowledge of physiologic races, is thus perhaps of some interest. The experiments described here fall into two series, separated in time. A preliminary investigation was started in 1932–4. This could not be brought to any certain conclusion, because seed of *Bromus arduennensis* and its variety *villosus*, regarded by Ward as particularly important bridging-hosts, could not be obtained. They were subsequently obtained and the investigation was begun again in 1940 and carried on until 1951. We feel that this lengthy experience enables us to discuss the stability of physiologic races of the brown rust of *Bromus* with some confidence.

EXPERIMENTAL MATERIALS AND METHODS

Host plants

The species of *Bromus* used were chosen to give adequate representation of the genus as it occurs in Europe. Ward (1902) divided the genus into five sections—Festucoides, Stenobromus, Serrafalcus, Libertia, and Ceratochloa. A similar division is generally accepted, but de Cugnac (1939*b*) has shown that the section Libertia, erected to contain *B. arduennensis*, should be dropped since a re-examination of the characters of *B. arduennensis* indicates that it is more correctly placed with *B. secalinus* in the section Serrafalcus.

We have referred our species to the sections Festucoides, Stenobromus, Serrafalcus, and Ceratochloa to facilitate comparison with Ward's results, though more recent authorities (Clapham, Tutin, and Warburg, 1952) have advanced these sections to generic rank with the generic names *Zerna* Panz., *Anisantha* C. Koch, *Bromus*, L., and *Ceratochloa* P. Beauv. respectively.

The species used, arranged in sections of the genus, with their provenance, are listed below. Those marked (*a*) were used in the first series of experiments, those marked (*b*) in the second series.

FESTUCOIDES

B. asper Murr. (*a*) National Institute of Agricultural Botany, Cambridge (N.I.A.B.), 1931; (*b*) collected near Cambridge by T. G. Tutin, August 1939.
B. erectus Huds. (*a*) N.I.A.B., 1929; (*b*) Botanic Garden, Cambridge (B.G.).
B. inermis Leyss. (*a*) B.G., 1931; (*b*) Botanical Gardens, University of Reading (Read.), February 1941.

STENOBROMUS

B. madritensis L. (*a*) Welsh Plant Breeding Station, Aberystwyth (W.P.B.S.), 1930; (*b*) Jardin Botanique de l'État, Brussels (Bruss.) 1936.
B. maximus Desf. (*a*) W.P.B.S., 1930.
B. sterilis L. (*a*) N.I.A.B., 1929; (*b*) Bruss., 1936.
B. tectorum L. (*a*) B.G., 1931; (*b*) University Botanic Garden, Uppsala, July 1941.

SERRAFALCUS

B. adoensis Hochst. (*a*) Botanic Garden, Bremen, 1929; (*b*) received as *B. patulus* Mert. & Koch from Bruss., 1936.
B. arduennensis Dmrt. (*b*) Bruss., December 1937.
B. arduennensis var. *villosus* (Strail) Crép. (*b*) Dr. A. de Cugnac, Paris, February, 1946.
B. arvensis L. (*a*) N.I.A.B., 1930; (*b*) Bruss., 1936.
B. brizaeformis Fisch. & Mey. (*a*) Read., 1932; (*b*) University Botanic Garden, Uppsala, July 1941.
B. commutatus Schrad. (*b*) collected at Hardwick, Cambs., by T. G. Tutin, August 1939.
B. grossus Desf. ex DC (*b*) Dr. A. de Cugnac, Paris, February 1946.
B. lepidus Holmberg (*b*) received from Read. as *B. racemosus* L.
B. mollis L. (*a*) W.P.B.S., 1929; (*b*) collected at Wort's Causeway, Cambs., by H. Gilbert-Carter, July 1941.
B. racemosus L. (*a*) Professor E. Foex, Versailles, 1932.
B. secalinus L. (*a*) N.I.A.B., 1931; (*b*) Bruss., 1936.

CERATOCHLOA

B. carinatus Hook & Arn. (*b*) B.G., 1936.
B. giganteus L. (*a*) N.I.A.B., 1931.
B. unioloides H.B. & K. (*a*) B.G., 1931; (*b*) Read., January 1941.
B. virens Büchl. (*a*) Botanic Garden, Munich, 1929.

Those species used in the second series of experiments, marked (*b*) above, with the exception of *B. asper*, *B. sterilis*, *B. arduennensis*, *B. arduennensis* var. *villosus*, and *B. grossus*, were critically examined for us by Mr. E. C. Hubbard, who confirmed the identification in all cases except two, mentioned above.

We were exceptionally fortunate in obtaining *B. arduennensis* and its variety *villosus*. The former is very rare and Professor Robyns of Brussels, who searched for it from 1932 onwards, did not obtain any until 1936. The variety *villosus*, according to de Cugnac (1939*a*, *b*), had become extinct early in this century. As a result of crossing *B. arduennensis* with *B. grossus* he obtained a true-breeding hybrid, in every way similar to *B. arduennensis* var. *villosus*, and he has suggested that where this used to occur it did so as a natural hybrid; it was, in fact, usually associated with the parent species.

Rust cultures

Each rust culture was maintained as a stock culture on a suitable host, such stock cultures being kept permanently under cellophane covers to prevent contamination. The origin of all cultures and the *Bromus* spp. used as stock hosts are given in a later section with the results.

Methods of inoculation

The methods of inoculation and techniques designed to prevent cross-infection were essentially the same as those described by Brown (1937). Plants were grown to the four- or five-leaf stage and the second or third leaf inoculated. The plants were grown in pots; in the first series of experiments 4 or 5 plants were grown in each pot and 1 or 2 leaves on each plant were inoculated; in the second series 2 plants were grown in each pot and only 1 leaf, usually the second, was inoculated.

In the first series of experiments not all the host species were inoculated at once and some such infected plants were used to inoculate further species, so that cultures on several different host species were used for inoculation. Consequently some species were inoculated more frequently than others but, with the exception of those cases noted in Table I, all results are based on the inoculation of at least 10 plants.

In the second series of experiments a complete set of hosts was inoculated on each occasion, always using spores from a stock culture. The composition of the set of hosts varied somewhat at first, but most species were used for over 30 inoculations, each of 2 plants. Exceptions to this were *B. unioloides*, which was not used more than 3 times, and *B. arduennensis* var. *villosus* and *B. grossus*, which were used for 17 inoculations in most cases. The data in Table II therefore are based on a considerable number of inoculations except in the case of *B. unioloides*; results with this species should be treated with some caution.

Inoculations were not carried out during the winter months (November to February inclusive) as normal reactions were not then obtainable.

Assessment of infection types

The plants were examined 14 days after inoculation and again some 4 or 5 days later for assessment of infection type.

In the first series of experiments, infection types were graded as follows: *immune* (—), no pustules formed, though necrosis was usually observed; *partially resistant* (+), small distinct pustules formed, with obvious necrosis and yellowing of the leaf; *susceptible* (++), large pustules formed with no necrosis and little yellowing.

In the second series, infection types were classified according to a scheme similar to that of Stakman and Levine (1922) as slightly modified by Brown (1937). The descriptions of the infection types according to this scheme are:

Host reaction.	Symbol.	Infection types.
Highly resistant	0	No uredospore pustules formed; necrotic or chlorotic flecks usually present.

Very resistant	1	Pustules minute, few and isolated; accompanied by pronounced necrosis.
Moderately resistant	2	Pustules small to medium in size but fairly numerous; surrounded by necrotic or markedly chlorotic areas.
Moderately susceptible	3	Pustules large and abundant; a little necrosis may be present, chlorosis usually present.
Very susceptible	4	Pustules large and abundant; no necrosis but sometimes slight chlorosis.

No X type of infection was seen.

RESULTS

First series

Table I summarizes the reactions of five cultures of brown rust on various host species of *Bromus*. In all cases single-spore cultures were established, but these gave reactions identical with the original isolations.

TABLE I

Reaction of Bromus spp. *to Infection by Five Cultures of Brown Rust*

	Culture				
Bromus species.	A.	D.	G.	H.	J.
asper	−		+	++	
erectus	−	−	−	++	++
madritensis	+	−	++		
maximus	−	−	++	++	
sterilis	++	−	++	+	+
tectorum	−	++	−		
adoensis	−	++	++		
arvensis	−	+	+		
brizaeformis	−	(−)	+	(−)	
mollis	−	++	++	++	++
racemosus	−	++	++		
secalinus	−	++	++	−	−
giganteus	−		−		
unioloides	−	−	+	−	
virens	−	−	++	(−)	

++ susceptible; + partially resistant; − immune

Note: reactions given in parenthesis are unreliable, being based on too few inoculations (< 10).

Culture A was obtained from uredospores on wild *B. sterilis* collected in Cambridge in May 1932; it was established first and maintained on *B. sterilis*. Of the various species inoculated, only *B. sterilis* and *B. madritensis* were infected, and the latter species showed considerable resistance, uredosori being always small. The pattern of infection was similar whether uredospores from *B. sterilis* or *B. madritensis* were used for inoculum. Two other cultures (B and C) also obtained from uredospores on wild *B. sterilis* in 1932 gave identical results and were discarded.

Culture D was obtained from uredospores found on *B. mollis* collected near Cambridge in May 1932; it was established first and subsequently maintained on *B. mollis*. This race has a wider host-range, all the species of the section Serrafalcus tested, except *B. brizaeformis*, being readily infected; the number of inoculations made on this last species were too few for its immunity to be certain. Of the infected species, *B. arvensis* was noticeably more resistant than the others. In addition to these, *B. tectorum*, of the section Stenobromus, was also susceptible to infection. Inoculations with uredospores produced on *B. mollis*, *B. secalinus*, *B. adoensis*, and *B. tectorum* gave identical results. Two other cultures (E and F), also obtained from uredospores on *B. mollis*, collected in May 1932, gave similar results and were discarded.

Culture G was obtained from uredospores on *B. maximus* collected by Professor Foex at Versailles in July 1932; it was established first and maintained on *B. maximus*. This had a still wider host-range, extending into all four sections of the genus. Experimental inoculations were made with uredospores from *B. maximus*, *B. mollis*, *B. sterilis*, *B. virens*, *B. madritensis*, *B. brizaeformis*, and *B. racemosus*, and in each case identical results were obtained.

Culture H was obtained from uredospores on *B. erectus* collected by Professor Foex at Versailles in May 1933. It was established first on *B. erectus*. Little work was done with this culture, but it was clearly distinct from those described above in producing vigorous infections of *B. asper* and *B. erectus*.

Culture J was obtained from aecidiospores from aecidia of *P. symphyti-bromorum* Müller on leaves of *Symphytum officinale* collected by Professor Fischer in Switzerland in June 1933. The few results obtained with this suggest that it may have been identical with culture H.

Many measurements of uredospore dimensions were made with all these cultures; they were biometrically indistinguishable. Teleutospores were only produced by cultures A and D. These were characteristic in size and shape of *P. bromina* Erikss., i.e. they were, in the terminology of Guyot (1937), brachysporous. Numerous attempts in 1933 and 1934 to infect *Symphytum officinale* and *S. peregrinum* with teleutospores from these two cultures were unsuccessful.

Second series

Characterization of physiologic races on differential hosts

The reactions of five cultures of brown rust on various species of *Bromus* are summarized in Table II. In all cases single-spore cultures gave results similar to those obtained with the original isolations.

Culture I was obtained from uredospores on *B. madritensis* collected in Cambridge Botanic Garden in July 1939. Stock cultures were maintained on *B. asper*. The results embody 6 sets of inoculations with the original culture and 31 with a single-spore culture. The host-range of this culture is unusual in that a few species from each section of the genus are highly susceptible whereas others are resistant or immune.

Culture II was obtained from uredospores on *B. secalinus* collected in

Cambridge Botanic Garden in July 1939. Stock cultures were maintained throughout on *B. secalinus*, except for a period of 1 year (1942–3) when it was kept on *B. lepidus*. The results embody 6 sets of inoculations with the original culture and 31 with a single-spore culture. The host-range is very similar to that of culture G of the first series of experiments, the main difference being the susceptibility of *B. tectorum* in this case. All species tested from the sections Serrafalcus and Stenobromus were susceptible. Four other cultures (Nos. III–VI inclusive), giving identical results, were isolated from uredospores on leaves of *B. arduennensis*, *B. commutatus*, *B. secalinus*, and *B. pratensis* respectively, all collected in July 1940, at the Rockefeller Field Station, Cambridge; these were discarded.

TABLE II

Reaction of Bromus spp. *to Infection by Five Cultures of Brown Rust*

	Culture				
Bromus species.	I.	II.	VII.	VIII.	X.
asper . . .	4	0–2	0	0	0
erectus . . .	0–2	0	0	0	0
inermis . . .	1–2	0	0	0	0
madritensis . . .	4	1–3	2–3	2–4	0
sterilis . . .	0–1	3–4	4	0	0
tectorum . . .	0–1	3–4	0–1	0*	0
adoensis . . .	0	3–4	0	0–4	0
arduennensis . .	0	4	0	4	4
arduennensis var. *villosus*	0	4	0	4	4
arvensis . . .	2–3	2	0	0–2	3–4
brizaeformis . .	0	3–4	0	3–4	3–4
commutatus . .	0	4	0	4	4
grossus . . .	3–4	4	0	4	4
lepidus . . .	4	4	0	4	4
mollis . . .	0	4	0	4	4
secalinus . . .	0–1	4	0	4	4
carinatus . . .	2–3	0	0	0	0
unioloides . . .	0	0	0	0	0

* On 3 occasions out of a total of 37 this species was infected: see text.

Culture VII was obtained from uredospores on *B. sterilis* collected in Cambridge Botanic Garden in July 1940. Stock cultures were maintained on *B. sterilis*. The results embody 9 sets of inoculations with the original culture and 28 with a single-spore culture. Only *B. sterilis* and *B. madritensis* were readily infected. This culture is probably the same as culture A of the first series of experiments. Similar cultures (Nos. IX and XI) were obtained from uredospores on *B. sterilis* collected at Cherry Hinton, Cambs., in July 1941, and near Edinburgh in October 1945. These cultures were discarded.

Culture VIII was obtained from uredospores on *B. mollis* collected at the Rockefeller Field Station, Cambridge, in August 1940. Stock cultures were maintained on *B. secalinus* until July 1942, and thereafter on *B. lepidus*. It was difficult to keep this culture alive in hot weather, but easier on *B. lepidus* than on *B. secalinus*. The results embody 11 sets of inoculations with the original culture and 26 with a single-spore culture. All species in the section Serrafalcus and one (*B. madritensis*) in the section Stenobromus were infected. This culture is very similar to culture D of the first series of experiments. The reaction on *B. adoensis* was characteristically very variable. On three occasions successful infection of *B. tectorum* was achieved; in one case this was due to accidental use of seed of the wrong species and, in another, mixture with another culture of the rust was suspected.

TABLE III

Reaction of Bromus spp. *to Culture II after Prolonged Subculture on* B. arduennensis

Bromus species	General reaction of stock on *B. secalinus.*	Number of subcultures on *B. arduennensis*											
		65	73	83	92	97	102	113	121	132	143	153	164
asper . . .	0–2	0	1	2	1	1	1	1	0	1	0	1	1
erectus . . .	0	0	0	0	0	0	0	—	0	0	0	0	0
inermis . . .	0	0	0	0	0	0	0	—	0	0	0	0	0
madritensis . .	1–3	3	3	2	3	3	3	3	3	3	2	0	2
sterilis . . .	3–4	4	4	4	3	3	4	4	4	4	4	4	4
tectorum . . .	3–4	4	4	4	3	3	4	3	4	4	4	4	4
adoensis . . .	3–4	4	4	4	4	4	4	4	4	3	4	4	4
arduennensis . .	4	4	4	4	4	4	4	4	4	4	4	4	4
arduennensis var. *villosus*	4	—	—	4	4	4	4	4	4	4	4	4	4
arvensis . . .	2	2	2	2	2	2	2	2	1	2	2	2	2
brizaeformis . .	3–4	4	4	3	3	3	4	4	3	4	4	4	4
commutatus . .	4	4	4	4	4	4	4	4	4	4	4	4	4
grossus . . .	4	—	—	4	4	4	4	4	4	4	4	4	4
lepidus . . .	4	4	4	4	4	4	4	4	4	4	4	4	4
mollis . . .	4	4	4	4	4	4	4	4	4	4	4	4	4
secalinus . . .	4	4	4	4	4	4	4	4	4	4	4	4	4
carinatus . . .	0	0	0	0	0	0	0	0	0	0	0	0	0

Culture X was obtained from uredospores on leaves of *B. arduennensis* collected at the Rockefeller Field Station, Cambridge, in June 1945. Stock cultures were maintained on *B. secalinus*. The results embody 3 sets of inoculations with the original cultures and 13 with a single-spore culture. This culture differed from culture VIII only in that no species outside the section Serrafalcus were susceptible.

The uredospores of all these cultures were indistinguishable. Measurements of teleutospores were not made.

Effect of repeated subculture on Bromus arduennensis

One of the 'bridging-species' on which Ward laid special stress was *B. arduennensis*. Only three of our cultures infected this species—Nos. II, VIII, and X, VIII and X being very similar. Nos. II and VIII were therefore repeatedly subcultured on *B. arduennensis* and at intervals tested out on the standard differential hosts and the reactions compared with the culture maintained on the normal stock host (*B. secalinus* for No. II, *B. lepidus* for

No. VIII). The results are presented in detail in Tables III and IV. Two points are worthy of note: (*a*) the remarkable similarity of reactions obtained at each of the twelve sets of inoculations, a constancy of reaction which characterized all our results; (*b*) even after 164 successive subcultures on *B. arduennensis* over a period of 8 years the reactions did not differ from those obtained with the normal stock culture. One abnormal result obtained with culture VIII from *B. arduennensis* on *B. tectorum* coincided with a similar anomaly, already mentioned, with the stock culture of this strain.

TABLE IV

Reaction of Bromus spp. *to Culture VIII after Prolonged Subculture on* B. arduennensis

Bromus species.	General reaction of stock on *B. lepidus*.	Number of subcultures on *B. arduennensis*											
		65	73	83	92	97	102	113	121	132	143	153	164
asper . . .	0	0	0	0	0	0	0	0	0	0	0	0	0
erectus . . .	0	0	0	0	0	0	0	—	0	0	0	0	0
inermis . . .	0	0	0	0	0	0	0	—	0	0	0	0	0
madritensis . .	2–4	4	4	3	3	3	4	3	2	3	3	3	3
sterilis . . .	0	0	0	0	0	0	0	0	0	0	0	0	0
tectorum . . .	0	0	0	0	0	0	0	0	0	0	0	0	4*
adoensis . . .	0–4	4	1	2	4	2	4	3	2	3	3	4	3
arduennensis . .	4	4	4	4	4	4	4	4	4	4	4	4	4
arduennensis var. *villosus*	4	—	—	4	4	4	4	4	4	4	4	4	4
arvensis . . .	0–2	1	0	0	0	1	1	1	1	2	1	2	2
brizaeformis. . .	3–4	4	4	3	4	3	4	3	3	3	3	4	4
commutatus . . .	4	4	4	4	4	4	4	4	4	4	4	4	4
grossus . . .	4	—	—	4	4	4	4	4	4	4	4	4	4
lepidus . . .	4	4	4	4	4	4	4	4	4	4	4	4	4
mollis . . .	4	4	4	4	4	4	4	4	4	4	4	4	4
secalinus . . .	4	4	4	4	4	4	4	4	4	4	4	4	4
carinatus . . .	0	0	0	0	0	0	0	0	0	0	0	0	0

* This anomalous reaction coincides with a similar one with the *B. lepidus* stock (see text).

DISCUSSION

Physiologic races and species of brown rust on Bromus

This work has provided evidence for the existence of the following physiologic races of brown rust on *Bromus*:

Race 1: cultures VII, IX, XI, and A, restricted to species in the section Stenobromus.

Race 2: culture X, restricted to species in the section Serrafalcus.

Race 2a: cultures VIII and D, similar in most respects to race 2 but also infecting occasional species of the section Stenobromus.

Race 3: culture I, infecting a few species in each section of the genus.

Race 4: cultures H and J, characterized by abundant production of uredospores on *B. erectus*.

Race 5: cultures II, III, IV, V, and VI, and probably also culture G, infecting species of the sections Serrafalcus and Stenobromus, *B. asper* in the section Festucoides and, in the case of culture G at least, some species of the section Ceratochloa.

So far in this paper we have not expressed an opinion concerning the nomenclature of the rust material we have used. We had in fact at first assumed that all could be considered to be *Puccinia bromina* Erikss. Recent French and Swiss workers, above all Guyot (1937, 1941), have considered that the brown rust of *Bromus* includes several species. We do not propose to comment on the usefulness of some of these recent subdivisions, but it may be of interest to consider our physiologic races in relation to this recent morphological work.

Guyot's main distinguishing criterion is the morphology of the teleutospore; a secondary criterion is the identity of the aecidial host, where known. He described two distinct morphological types and includes both in the group-species *P. dispersa* Erikss. & Henn. *sensu lato*. One of these types has short teleutospores with both upper and lower cells relatively broad; this he calls the brachysporous type. He describes two species of this type as native to Europe—*P. bromina* Erikss. and *P. madritensis* Maire. The other type has elongated teleutospores, with both cells, and particularly the lower cell, very narrow; this he calls the dolichosporous type. Rusts of this type native to Europe are *P. bromi-maximi* Guyot, *P. alternans* Arthur f. sp. *bromi-erecti* Gäumann, and *P. alternans* f. sp. *bromi-benekeni* Guyot. In addition there is yet another species of interest to us—*P. symphyti-bromorum* Müller. Many authorities have considered this to be synonymous with or included in *P. bromina* Erikss., although no aecidial host has been found for many strains of *P. bromina*. At the time Guyot was writing (1941) the teleutospore shape of this species was uncertain, though, as Guyot pointed out, if Müller's original diagnosis was correct, it had dolichosporous teleutospores, not brachysporous as in *P. bromina*. More recently, further information has become available, unfortunately making the position even more confusing. Gäumann (1941) and Dupias (1951) have described three formae speciales of *P. symphyti-bromorum*: f. sp. *typica* (the characteristic form on *B. erectus*), f. sp. *benekeni*, and f. sp. *tuberosi-asperi*, differing in their aecidial and uredinial host-ranges. The last two forms, it has now been found (Dupias, 1951), have brachysporous teleutospores like *P. bromina*, but the first has, as Müller described it, dolichosporous teleutospores (Mayor, 1951). The present confused state can only be settled by a combination of morphological studies with host-range studies such as we have described, using material collected throughout Europe.

All our cultures fall into *P. dispersa* Erikss. & Henn. *sensu lato*. Races 1 and 2 can confidently be placed in *P. bromina* Erikss. Race 4 is probably (culture J certainly) *P. symphyti-bromorum* Müller f. sp. *typica* Gäumann. It is not possible to speak with any degree of certainty about the others, but it is possible that culture G (race 5) may be *P. bromi-maximi* Guyot, judging by the host-range. In the uredo-stage all are morphologically indistinguishable

The bridging-host theory

If it is true that we may have been working with more than one species of Brome rust, as the species are understood by Guyot, it is equally true of Marshall Ward's work. We have, however, defined our physiologic races by their reactions on a series of carefully determined differential host species of *Bromus*, and that is the most relevant matter to a consideration of our results in relation to the bridging-host theory.

In the work described in Ward's first paper (1902) he was dealing with two physiologic races, corresponding to our races 1 and 2. That is to say, he obtained from *B. sterilis* a race infecting only species of the section Stenobromus, and from *B. mollis* a race infecting only species of the section Serrafalcus. The rare exceptions to this generalization are explicable, as indeed he explained them, by accidental infection, since he took far fewer precautions to avoid this than modern rust investigators do. He had not at this time formulated his bridging-host theory, but the results obtained in his early work led him to a conclusion which affected the development of the theory. This conclusion was that the host-range of the rust was determined by the taxonomy of the host. This he expressed on one occasion (1905) as follows: 'in the vast majority of cases the uredospores from a given species of *Bromus* only infect the same species or, usually in diminishing proportions, species closely allied to it and in the same group (of the genus)'. This is very true of our races 1 and 2, the two races with which he was exclusively working in the first place. Had he in his early work encountered our races 3 and 5, whose host-range is unrelated to the taxonomy of the genus, his conclusions might have been different. If the host-ranges of all our races are considered, it is clear that the taxonomic theory of host-range is untenable.

A detailed examination of the results of Freeman (1902) and the later results of Ward (1903) strongly suggests that they subsequently collected other races of the rust with a less restricted host-range. For instance, Ward (1903) reports results with a culture obtained from *B. crinitus* which readily infected species in the sections Stenobromus, Serrafalcus, and Ceratochloa; this appears to have been similar to our race 5. A form which he obtained from *B. arvensis* showed a similar host-range. Both Ward and Freeman appeared to be unaware of the possibility that they might be dealing with a new physiologic race and were led to attribute this infection of hosts previously immune, or infection of a wider range of hosts, to an adaptive change in the infective potentialities of the rust. Many, if not all, of the anomalous results discussed by Ward (1903) can be explained by the unconscious use of new and unrecognized races. All their infective material was apparently collected either from wild plants or from plants growing without protection in the Botanic Garden, Cambridge, and no attempt was made to ensure their purity, so that mixture of races may also have entered into the production of their anomalous results.

The case of *Bromus arduennensis* requires more detailed consideration, since Ward (1903) attached so much importance to it. He first noted that the race of rust obtained from *B. mollis* (our race 2) readily infected both *B. arduennensis* and its variety *villosus*. This in itself struck him as remarkable, since he regarded *B. arduennensis* as belonging to a distinct section of the genus (Libertia), and according to his taxonomic view of host-range determination this was not to be expected. De Cugnac (1939) has presented cogent evidence for believing *B. arduennensis* to be closely related to *B. secalinus* and, therefore, to be placed in the section Serrafalcus. Accordingly, since race 2 appears to be infective to all members of that group, infection of *B. arduennensis* is to be expected. Even if it were to be placed in a distinct section of the genus the result could not be considered anomalous since we have now many examples of physiologic races infecting taxonomically distant species.

Ward (1903) was still further impressed by the fact that *B. arduennensis* var. *villosus* was infected both by the rust from *B. mollis* (our race 2) and by a race which he isolated from *B. sterilis*. He did not inoculate the latter culture on sufficient host species for it to be properly characterized, but it was probably not the same as our race 1, since we have found both *B. arduennensis* and its variety *villosus* resistant to that race. His result only indicated that the two races had a common host in *B. arduennensis* var. *villosus*. This is not an uncommon occurrence, as we have seen, but Ward did not expect such overlapping of host-range. He was led from this observation to conclude that 'we may suppose a uredospore from *B. sterilis* to infect *B. arduennensis* var. *villosus* and the crop of spores produced on this to further infect *B. arduennensis*; thence the fungus could pass to *B. secalinus* . . .' (Ward, 1903). Nowhere does he record ever having attempted to confirm this experimentally. We have reported several instances of a race of rust being grown on several hosts, yet producing on each host spores with similar and unchanged host-range. In particular, we have shown that repeated subcultures of two races on *B. arduennensis* was completely without effect on their host-range. After 164 subcultures on *B. arduennensis*, culture VIII (race 2*a*), originally obtained from *B. mollis*, was no more able to infect *B. sterilis* than when it was first isolated. We must conclude that our experience with Brome rusts confirms the general experience of all recent workers on cereal rusts and that the bridging-host theory must be abandoned. We do not in any way wish to deny the intimacy of the host-parasite interaction, but do maintain that under normal conditions physiologic races of the Brome rusts, as of other rusts, are essentially stable in their host-range.

SUMMARY

1. Nineteen cultures of the brown rust of Brome grasses have been isolated and studied in pure culture, a number of them as single-spore strains. Their infection reactions on a series of *Bromus* spp., representing all four sections of the genus (Festucoides, Stenobromus, Serrafalcus, and Ceratochloa), were observed over a period of 14 years, and used to characterize physiologic races.

2. Five physiologic races have been distinguished. *No. 1* was restricted to species in the section Stenobromus. *No. 2* was restricted to species in the section Serrafalcus. A race differing from the latter only in also infecting occasional species in the section Stenobromus was called *No. 2a*. *Nos. 3, 4,* and *5* had a wider host-range infecting species in three or more of the four sections of the genus, *No. 4* being the only one to infect *Bromus erectus*.

3. In the uredospore stage all these races were morphologically identical. The teleutospores were not studied, so it is not possible to refer all these strains definitely to species as understood by some recent workers. All belong to the group-species *Puccinia dispersa* Erikss. & Henn. Judging by their host-range the species *P. bromina* Erikss. (*races 1 and 2*) and *P. symphyti-bromorum* Müller (*race 4*) were represented; possibly also represented was *P. bromi-maximi* Guyot (*race 5*).

4. All physiologic races have been remarkably stable in their infection-types and host-range. No observations have been made lending support to Marshall Ward's 'bridging-host' hypothesis. Two physiologic races (*2a* and *5*) were cultured for 164 generations, over a period of 8 years, on *Bromus arduennensis*, a species regarded by Ward as of special importance as a bridging-host. At the end of this period the races were indistinguishable in their host reactions from the original culture from which they were obtained or from stock cultures maintained during the same period on *B. lepidus* or *B. secalinus*. It is believed that all the results used by Ward to support his theory can be explained by his having worked with more physiologic races than he recognized, particularly with those of wider host range, and by lack of precautions to prevent mixture of races.

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The 'Diffuse' Centromere or Polycentric Chromosomes in *Spirogyra*

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With Plates V and VI and four Figures in the Text

ABSTRACT

1. Among ten species of *Spirogyra* types are distinguished with large-, medium-, and small-sized chromosomes.

2. Evidence of compound structure is presented with regard to the large- and medium-sized chromosomes.

3. Parallel separation of chromatids, first described by Geitler for *S. crassa*, is found in all species having large chromosomes and to some degree in those with medium-sized chromosomes.

4. It is suggested that the parallel separation of chromatids is caused by their polycentric nature.

5. Polyploidy in the genus is only doubtfully comparable with that in the higher plants.

6. It is suggested that in *Spirogyra* the localized centromere may have evolved in a polycentric chromosome.

7. Comparisons are drawn between *Spirogyra* and other organisms stated to have 'diffuse' centromeres and it is suggested that their chromosomes also are polycentric.

INTRODUCTION

IT is 23 years since Geitler described the first example of parallel separation of chromatids at mitosis in a plant, the alga *Spirogyra crassa* (1930). He attributed this to the fluidity of the spindle or the rigidity of the chromosomes. A similar phenomenon in the Coccid *Protenor* was attributed by Schrader in 1935 to the presence of a 'diffuse' centromere; many Coccids are now known to resemble *Protenor* in this (see Hughes-Schrader, 1948). In 1947 Malheiros, Castro, and Camara described the second example of parallel chromatid separation in the plant kingdom, for *Luzula*, adopting the explanation of the 'diffuse' centromere; the description of the chromosomes of *Carex* by Wahl in 1940 indicates that they are probably similar. Nordenskiöld (1951) suggests that the same type of chromosome is probably found in *Scirpus* and *Juncus*; she, too, accepts the explanation of the 'diffuse' centromere. Hughes-Schrader (1948) in her review of Coccid cytology suggests that the chromosomes of Homoptera, Heteroptera, and many Lepidoptera may resemble those of Coccids in possessing the 'diffuse' centromere.

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An alternative explanation of parallel separation of chromatids is, of course, that the chromosomes are polycentric, as in the well-known case of *Ascaris megalocephala*. Hughes-Schrader says (1948, p. 132): 'To assume that it [the chromosome] is compound or multiple in nature is unjustified so long as no repetition of structural units can be demonstrated.' The present paper gives evidence of repetition of structural units in the chromosomes of *Spirogyra*, and draws attention to similarities other than parallel separation of chromatids between the chromosomes of *Spirogyra* and those of the other organisms referred to above.

Something can be seen of spiralization in the bigger chromosomes of *Spirogyras* and this is described. So far as the author is aware no information regarding spiralization is available for the other organisms.

THE DIFFERENT TYPES OF CHROMOSOME IN THE GENUS

Examination of mitosis in many species has shown that parallel separation of chromatids is found in all species having large chromosomes and to some extent in those with smaller ones. Species exist, however, in which there is a localized centromere, frequently nearly terminal in position; and others in which the chromosomes are so small that the position of the centromere is immaterial.

The metaphase chromosomes in the different species fall into the following four main types:

Type 1. The chromosomes are characterized by large size, sinuous shape, and sticky matrix; the chromatids are parallel at anaphase, separate at metaphase throughout their length; the centromere is 'diffuse'.

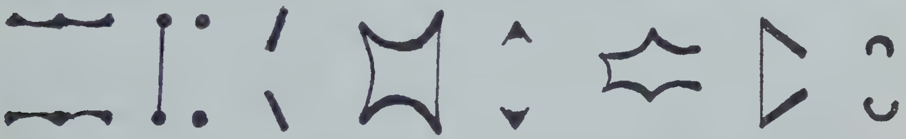
S. sub-echinata (Godward, 1953; in press), *S. crassa* (Godward, 1950, 1953), and *S. triformis* (ibid.) possess chromosomes of this type (Pl. VI, Figs. 2, 3, 5). The length ranges from 5 to 10 μ and the breadth from 0·5 to 1 μ. The sticky matrix is illustrated elsewhere (Godward, 1953) for *S. triformis*. It is seen when the chromosomes are lying close to one another on the plate and is found using any fixative. The nature of this 'matrix' which obscures to a great extent the structure of the metaphase chromosomes is in part discussed elsewhere (ibid.). 'Matrix' is a word of questionable significance when used for this sticky outer coating of the chromosomes, as has been shown (Darlington, 1937), but it is used here to facilitate comparisons with other organisms where a similar coating has been termed by authors 'matrix'.

The sinuous shape of the chromosomes is shown in Pl. VI, Figs. 2, 3, 5, also in Godward, 1953, Fig. 3, and it reflects the absence of any localized centromere such as would reveal its presence by the formation of a sharp bend in the metaphase chromosome.

The most striking characteristic of these chromosomes, already published in note form (Godward, 1950) and mentioned in the Introduction, is the parallel separation of the chromatids at anaphase (Pl. VI, Figs. 6, 7, 8), no part of the chromatid preceding any other part to the pole, as a general rule.

This was already observed in 1930 for *S. crassa* by Geitler. In the plant kingdom it is interesting to note the existence of such an unusual type of chromosome in such widely different organisms as the Angiosperms *Luzula* and its relatives on the one hand; and *Spirogyra* on the other; to the latter can now be added its relatives, the desmids *Pleurotaenium* and *Cosmarium* (King, 1953). It is clear that there is no localized centromere which has remained undivided till metaphase as normally happens, since from the first appearance of the metaphase chromatids these are separated from one another from end to end.

It is noteworthy that there is one region of the chromosome which does not lie parallel with the rest and with the equator of the spindle at anaphase, but hangs behind and lies in line with the spindle-fibres. This is the nucleolar-organizing region of the N.O. chromosome (usually there are two such chromosomes), slightly subterminal in position and carrying with it its satellite. This part of the chromosome then may be presumed to contain no structure having the activity or nature of a centromere (see Godward, 1950, and 1953, Figs. 4 and 6). So far as I am aware no such behaviour of part of a chromosome has been reported in the other organisms having a 'diffuse' centromere.



TEXT-FIG. 1. Separation of chromatids at mitotic anaphase, *S. sub-margaritata*. The separation varies from parallel to normal in the different types of chromosome, which seem to show the presence of one, two, or three centromere units.

Type 2. Here the chromosomes are of medium and small sizes, and without stickiness; parallel and normal separation of chromatids are found in the same chromosome set, i.e. the 'diffuse' and the localized centromere are both found.

S. sub-margaritata (Godward, in press) is the only named example although others have been seen. Text-fig. 1 shows the chromosomes. The sizes are 1 to 3 μ in length. Some of the parallel-separating chromatids seem to show evidence of two points proceeding slightly ahead to the pole, as if there might be two centromeres. Where there seems to be a medianly located centromere the shape of the small chromatids with the medium point going to the pole is reasonably convincing evidence of it. Other chromosomes are too short and dot-like to afford any evidence regarding the centromere.

It seems quite possible that in this species we have evidence of the compound nature of the chromosomes with parallel-separating chromatids. Whether fusion or fission of chromosomes has occurred cannot be said. The 'diffuse' centromere here would then not be a likely interpretation of the facts; rather the chromosomes presumed to show it would be polycentric.

Type 3. This has chromosomes of medium size with no stickiness; chromosomes with localized centromere, usually sub-terminal.

The length of the metaphase chromosomes in the example *S. brittannica* ranges from 2 to 5 μ and the width from 1 to 0·5 μ. They are much more attenuated at anaphase when the position of the centromere becomes evident (Text-fig. 3; and Godward, 1953, Pl. XXIII, Fig. 3). A striking contrast to the anaphase of the species of type 1 is seen here in type 3, where all the long chromosome arms lie in line with the long axis of the spindle. Most of the chromosomes seem to have the centromere almost terminal; only one or two of the chromosomes show it clearly subterminal or median (Pl. V, Figs. 7 and 8). Another example is an unidentified species to which the temporary designation of *S.F.* has been given.

The longest chromosomes are the N.O. chromosomes, and, as in all species, these hang prominently behind the rest at anaphase (see also Godward, 1953).

Type 4. Here chromosomes are of small or minute size and there is no stickiness. There is no possibility of seeing the position of the centromere on the chromosome.

Most species of *Spirogyra* are of this type. Those which have been identified or given a temporary designation are *S. daedalea*, *S. maxima*, *S. gracilis*, *S. oblata*, S.L.S., S.H.3 (see Godward, in press).

THE NATURE OF THE PROPHASE CHROMOSOME

Type 1. It appeared when *S. crassa* was examined by the writer in 1945 that the mitotic prophase was remarkable in that the chromosomes first made their appearance as a large number of apparently discrete dots, which later could be seen to present an arrangement in linear series. A paler staining material apparently was between the deeply staining dots or blocks, so that the chromosomes looked as though they had large deeply staining chromomeres (Pl. V, Fig. 5). This appearance was lost with further contraction of the chromosomes towards metaphase. In *S. crassa* the numbers of these deeply staining blocks was too large to permit of accurate counting, nor was it possible to match the arrangement on one chromosome with that on another. In *S. triformis* the numbers were somewhat smaller and there was some suggestion of a similar arrangement on each of a pair of chromosomes. It was suggested to me (L. La Cour) that the appearance of 'chromomeres' might be due to the fixing and staining methods used (Godward, 1950) and that this appearance was really due to breaks in the chromosomes made during squashing. In this connexion it is perhaps of interest that the same appearance of discontinuous staining of early prophase chromosomes in *S. crassa* appears in the figures of Moll (see Godward, 1950) and Geitler (1930), whose methods were different from mine. However, a species was eventually found (*S. subechinata* G., see Godward, in press) in which, by good fortune, the numbers of the deeply staining blocks or 'chromomeres' was small. Pl. V, Figs. 1–4, show early and mid-prophase in this species.

Here there are only four chromosomes. Of these, two are nucleolar-organizing chromosomes. These show, at mid-prophase (Pl. V, Figs. 1 and 2) a distal block (the satellite), then the N.O. region, then two blocks close together, a less stained region, then two blocks less close than the first two with a short less stained region between them, then two more blocks well separated from the last two and each other by less stained regions. The other two chromosomes each show five blocks equally separated by less stained regions. One could hardly ask for clearer evidence to show (1) that the appearance is not due to breaks; (2) that the number and arrangement of the blocks is constant for the chromosome type; (3) that there are two homologous pairs of chromosomes; (4) that the chromosomes are made up of two sorts of material, deeply staining and less staining. In addition both figures show what may possibly be somatic pairing of homologues.

First one may consider the numbers of the blocks. In these mid-prophase the N.O. chromosomes each have 7, the others 5, giving a total of 24 items At earlier prophases (Pl. V, Fig. 3), where the blocks are not visibly connected, 24 can be counted in the preparation. A series of ultra-violet photographs, taken at × 1,000 through a nucleus at very early prophase, showed 32 such items of which some were very small. It is clear, then, that the number is rather larger than 24, but 24 will usually be seen by examination with visible light. It seems possible that the number may be reduced to 24 from 30 by contraction of the intervening material in early prophase. The ultra-violet photograph at early prophase showed little sign of material connecting the blocks—only at one place a fine straight fibre is seen connecting them. It showed no structure in the blocks themselves.

Below are given approximate counts for numbers of stained blocks seen in very early and early prophase in *S. crassa* and *S. triformis* also.

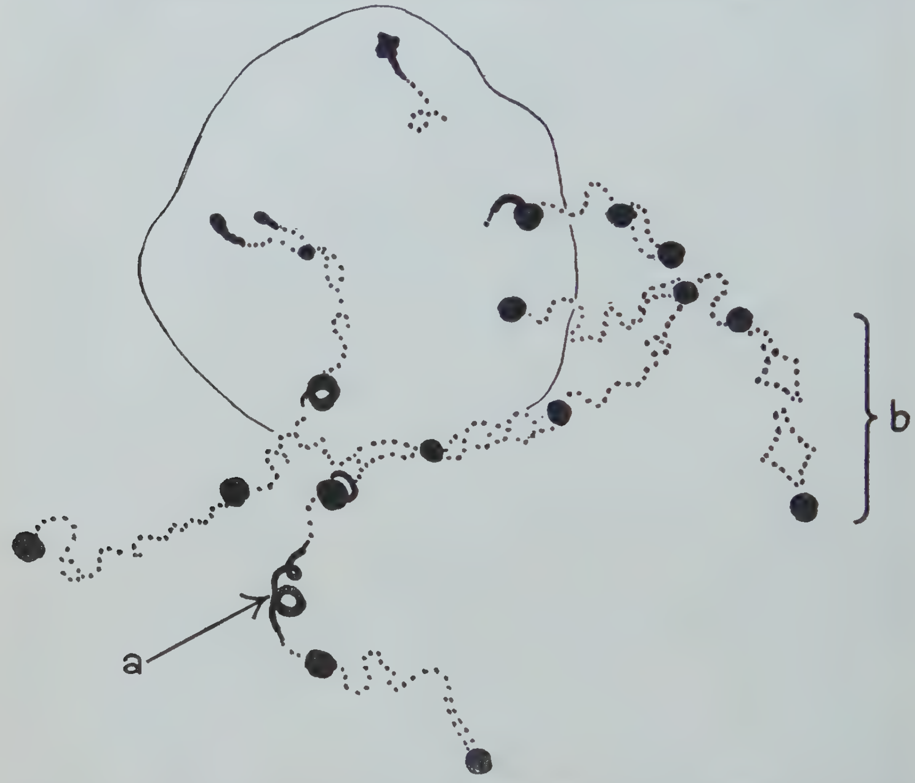
	Number at earliest prophase.	Number at early prophase
S. crassa . . .	356	258, 229
S. triformis . .	No estimate	165, 161
S. sub-echinata . .	31, 32	24

THE METAPHASE CHROMOSOME; SPIRALIZATION

Undoubted spiral structure can be seen in the chromosomes of *S. crassa*, *S. triformis*, and *S. sub-echinata*, at mid- and late-prophase and at metaphase; the expanded remains of these spirals can be seen at anaphase. (Pl. VI, Figs. 1–4.)

It is difficult to draw comparisons between these spirals and those found in other members of the plant kingdom. Spirals as large as these (Pl. VI, Fig. 3) are not usually found at somatic metaphase. This alone would tend to suggest that these are not exactly comparable with somatic spiral structure as usually understood. The existence of a 'molecular' spiral is of course assumed.

In prophases (Pl. V, Fig. 4) the two chromatids are seen at times, not very frequently; giving the impression that they are wound up together. From the shape of the coil which apparently includes both chromatids, one would not be inclined to say it was the relational coil. Yet such a relational coil should presumably exist, unless the peculiar nature of the chromosome in regard to its centromere organization precludes its development. It may, of course, have already straightened at an earlier prophase stage. The coils which can be observed, somewhat obscurely (Pl. V, Fig. 4, and Pl. VI, Fig. 3; see also Godward, 1950), are present in both the pale staining and deeply staining—perhaps one can say euchromatic and heterochromatic—parts of the prophase chromosome (Text-fig. 2). An idea of the dimensions is given below.



TEXT-FIG. 2. Camera lucida drawing of a prophase stage of *S. sub-echinata*. At *a* is seen spiralization in a heavily staining region. The dotted regions between the stained blocks are pale staining and show coiling, also in places the presence of the two chromatids, which would appear to be coiled around each other. The section *b* is shown in Pl. V, Fig. 4.

Number of coils in a chromosome of *S. triformis*

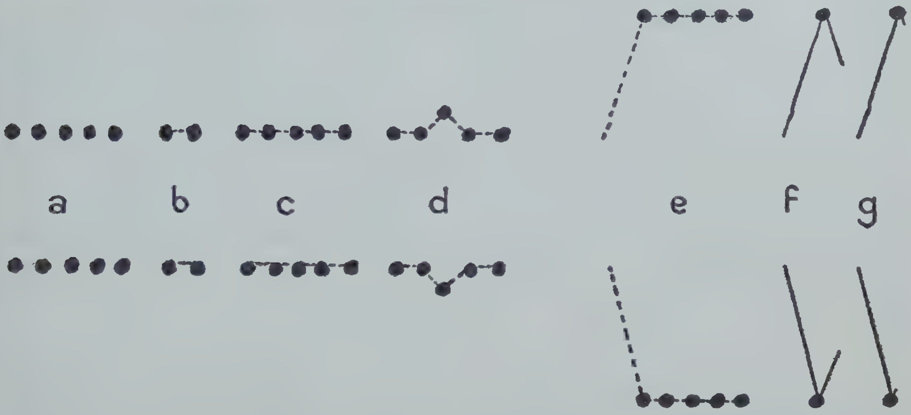
At mid-prophase (Godward, 1950, Pl. V, Fig. 5, lowest right-hand chromosome) 11 in 10μ

At metaphase (this present paper Pl. VI, Fig. 3) 5 in 5μ

It seems probable that there are fewer coils per unit length of chromosome in

S. sub-echinata. The spiral, such as it is, it characterized by its coarseness and irregularity.

In Pl. VI, Figs. 2 and 3, are printed from the same negative, heavily (Fig. 2) to show the outline of the metaphase chromosome, largely 'matrix', and lightly to show the internal spiral. When this is compared with the anaphase of the same species (Pl. VI, Fig. 1) it is clear that the internal spiral of the metaphase chromosome has a close relationship with the shape of the anaphase chromatid. This has a sinuous shape which could be called a loose spiral but that it lies in one plane. The sister chromatids are identical in shape (Pl. VI, Figs. 1 and 4). This shape then is laid down at the first separation of the sister chromatids. How can the single coarse spiral including both chromatids be transformed into two 'flat spirals', one lying on top of the other, without much apparent change in the look of the spiral itself when seen from polar view? The stages of this separation, if they do occur, are obscured by the 'matrix'. It is to be noted that the chromatids are not apparently separated in Pl. VI, Fig. 3.



TEXT-FIG. 3. Scheme showing the presumed structure of the various chromosome types in *Spirogyra* species, shown at anaphase. It is emphasized that this scheme is merely a tentative interpretation. At *a*, small dot-like chromosomes (*S. oblata*); *b*, two dot-like units joined (*S. sub-margaritata*); *c*, several units joined giving parallel separation (*S. crassa*, &c.); *d*, one unit predominates over the others (*S. sub-margaritata*, *S. brittannica*) giving a localized centromere in a large chromosome; *e*, a chromosome of the type of the N.O. chromosome of *S. crassa*, showing the N.O. region devoid of centromeric activity; *f*, *g*, chromosome types with localized centromere at late anaphase (*S. brittannica*).

Size relation between anaphase and metaphase. The following data are of interest:

S. triformis: Length of longest (N.O.) metaphase chromosome 10 μ
Breadth of metaphase plate 15 μ
,, ,, late anaphase plate 12 μ

S. crassa: Length of longest (N.O.) metaphase chromosome 9·2 μ
Breadth of metaphase plate 20–30 μ
,, ,, anaphase 11·2 μ
Length of longest anaphase chromosome 9·2 μ (not taking the curves into account)

Metaphase and anaphase chromosomes of both *S. triformis* and *S. crassa* are seen in Pl. VI, Figs. 1 and 4. The change in the chromosomes at anaphase seems at first sight to consist mainly in the loss of stainable material (see Godward, 1953) which can be seen sloughing off them in Pl. VI, Figs. 1 and 4, and in the expansion of the chromatid which is passing into telophase. The measurements show, however, that there is little increase in the length of the chromosome-chromatid, while there is a great contraction of the anaphase plate as compared with the metaphase.

At the earliest anaphase (Pl. VI, Figs. 6 and 7) the discontinuity in the appearance of the chromatid is visible, which one could associate with a presumed compound nature; these chromomere-like swellings may probably only indicate the first discharge of stainable material from the chromatid in its first steps towards anaphase (see Godward, 1953). In the Coccids similar swellings are described as 'interzonal connections' (p. 132). It does not seem that any information on spiralization is available for other organisms with the 'diffuse' centromere. In *Spirogyra* it is only in the large chromosomes of type 1 that anything of the kind has been seen.

STRUCTURE OF THE STAINED BLOCKS

In *S. sub-echinata* one or two stained blocks have been observed to extend over a sufficient length of the prophase chromosome to show spiralization within the block (Text-fig. 2). The same has been observed in *S. triformis*. Usually, however, one may not see even the chromatids within the block, and in the earliest prophase of *S. sub-echinata* it appears as a structureless sphere (Pl. V, Fig. 3). Since eventually the entire chromosome becomes stainable and may at times show spiralization throughout its length, the stained blocks are thus merely parts which show staining earlier than the rest. This is the typical appearance of the region adjacent to the centromere as recorded by Heitz (1929) and others, and, as in those cases, these regions correspond with the stained 'chromocentres' of the resting nucleus.

CHROMOCENTRES

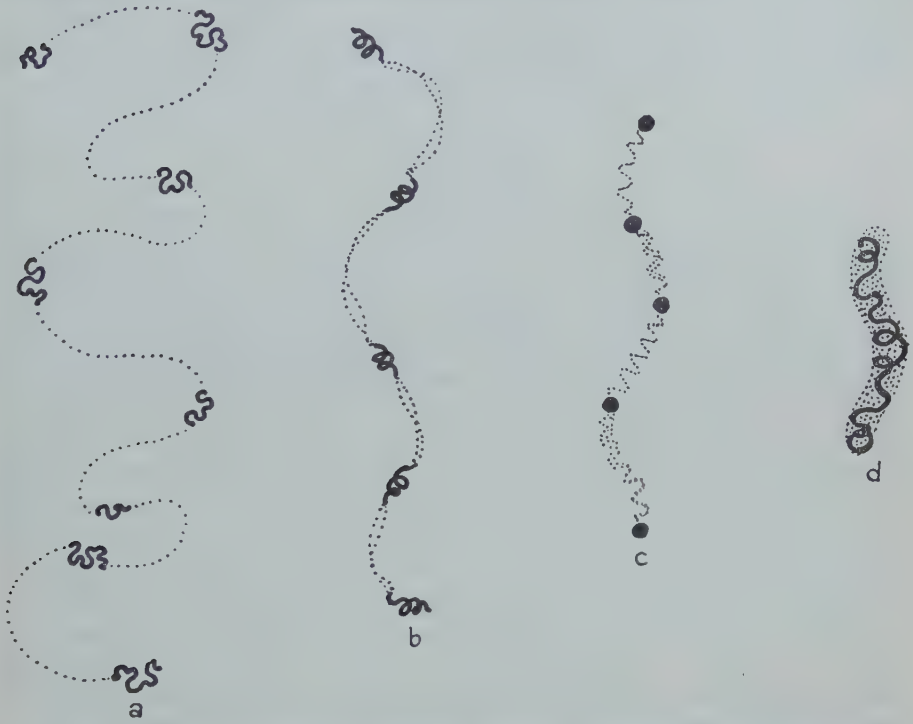
As telophase is entered on, the expanding chromosome again comes to consist of stained blocks with intervening unstained regions, and these regions finally expand into the chromocentres found near the periphery of the resting nucleus. These, especially in *S. sub-echinata*, have an irregular looped form, presumably relic spiralization.

The transition from resting stage to metaphase. Text-fig. 4 is a schematic representation of a chromosome, e.g. of *S. sub-echinata* at resting stage, earlier and later prophase, and metaphase. The scheme is limited, since it is not possible to make observations regarding several important features of structure. It shows that the stained blocks of prophase are derived from the chromocentres of resting stage, after fusions during contraction; the spiralization, the chromatids, the stained blocks or heterochromatic regions of prophase;

the coarse spiral, the disappearance of chromatids, and the 'matrix' of metaphase.

FRAGMENTATION OF THE CHROMOSOMES

It was hoped to fragment the chromosomes experimentally using X-rays or ultrasonics. However, nearly 3 years have passed since one of the suitable species was last collected in the natural habitat and it has not been found possible to maintain them indefinitely in culture. Tentative experiments failed to give any evidence of chromosome breaks, but this may have been due to the unsuitability of the apparatus. However, there was ample evidence of spontaneous fragmentation both in nature and in culture. In *S. crassa* a naturally occurring strain had one N.O. chromosome with a very long N.O. region, the other with a very short one, instead of two N.O. chromosomes with equally long N.O. regions. Here presumably fragmentation had been followed by translocation. In *S. sub-echinata*, normally having four chromosomes,



TEXT-FIG. 4. Scheme showing the presumed course of prophase in *S. sub-echinata* and the other species having similar chromosomes. At *a*, the resting stage or earliest prophase; the relic spiral is seen, the dotted line indicates the non-staining and invisible material between the stained blocks or chromocentres; *b*, the stained blocks show an irregular coil, the intervening regions which now stain palely a loose (perhaps the relational) coil; both chromatids are seen at places; *c*, the stained blocks are contracted and dense, the intervening material stains more, and there is a closer irregular coil; chromatids are not now distinguishable; *d*, the metaphase, with a wider irregular coil and 'matrix'. No distinction between stained blocks and intervening regions can be made.

a strain with one very long and one very short chromosome, and a strain with five (two short) chromosomes, arose in culture. In all cases the abnormal chromosomes behaved exactly like the others in the mitotic spindle.

The strains which arose in culture are of particular interest, since the change must have come about during mitosis. Breaks, with or without reunions, might easily occur during the long prophase when what are here presumed to be 'connecting regions' may be weak and entanglements, frequently observed in *S. sub-echinata*, perhaps complicated by somatic pairing (Pl. V, Figs. 1 and 2), might lead to such results. If this were to be so it would indicate the presence in the prophase chromosome of regions capable of breaking and rejoining more readily than is normally expected.

POLYPLOIDY

Bearing in mind that we have in the genus several types of chromosomes, viz. compound (or with diffuse centromere), with localized median or subterminal centromere, and dot-like where the position of the centromere cannot be determined; and also the possibility that the dot-like chromosomes may be equivalent to parts of the compound chromosomes, we may doubt whether polyploidy as it is understood in the higher plants is to be found here.

The first possibility which may be considered is that the species with large, compound chromosomes (e.g. *crassa*, *triformis*, *sub-echinata*) are polyploid. Of these, *S. sub-echinata* provides highly convincing evidence that its four chromosomes are two homologous pairs; in *S. triformis* there is a strong possibility that the six chromosomes are three homologous pairs (see Godward, 1950); in *S. crassa* there are two apparently identical N.O. chromosomes but the other ten have no distinguishing characteristics (Pl. VI, Fig. 5). The proof would, of course, be found in meiosis, but the critical stages are unfortunately not yet to hand, although the four nuclei resulting from meiosis in *S. crassa*, three of them degenerate, have been seen. There is no doubt that meiosis takes place, if conjugation is, as it appears to be, effective. The conjugating thread therefore would be diploid, the thread tetraploid.

We have now to consider those other species, by far the majority, with numerous small or dot-like chromosomes, some of them showing evidence of compound structure but consisting only of a few units, most of them being apparently single centromere-units. The bulk of these species have two N.O. chromosomes; some have more but none has one only, as expected of a haploid. These also could therefore be regarded as diploid with a tetraploid zygote. If the chromosome number, however, depends on the degree of combination, or lack of it, of the small one-centromere chromosomes we should expect to find that the various species exhibit a great range of chromosome numbers, the numbers being without relationship to one another. This is, so far as present evidence goes, the actual situation. Polyploidy in these types, if the conception of polyploidy is of any significance here, would not be indicated by the actual chromosome number, but by the number of N.O. chromosomes.

A few species, as has been mentioned, have more than two N.O. chromosomes. Four N.O. chromosomes, found in two species so far, might indicate a still higher degree of polyploidy, but there is no evidence that all four are homologous, though there is none against this supposition. Three N.O. chromosomes, found in two species so far, might be the sign of a triploid (hexaploid zygote). One feels, however, that there is little point in adopting any very definite ideas on polyploidy in the genus until more facts are available.

Cytotaxonomy. Does a species have a constant chromosome number and constant cytological characters, distinguishing it from others? This question, which suggests itself, may be answered in the affirmative, on present evidence. The cytotaxonomy of the genus will be the subject of another paper (Godward, in the press, J. Linn. Soc.), the first of a series.

DISCUSSION

This must centre on the alternatives—'diffuse' centromere or polycentric chromosome. There is no positive evidence regarding the appearance of the centromere itself in *Spirogyra*, any more than in the other organisms concerned. It is necessary therefore to argue from behaviour and from analogy. In *Spirogyra* the following points may support the view that the chromosomes are polycentric.

1. In long chromosomes there is discontinuous staining at prophase, each 'stained block' corresponding to one or more chromocentres of resting stage. Precocious staining of similar regions corresponding to chromocentres, and representing regions on either side of a localized centromere, has been shown by Heitz (1929), the name 'chromocentre' expressing the significance of the region concerned. Thus the stained blocks of *Spirogyra* could represent regions containing a centromere. They are constant in number and arrangement.
2. In medium-sized chromosomes the presence in some of apparently 2 or 3 centromeres, as shown by the shape at early anaphase; the presence of a localized centromere in others. Here there is a suggestion that 2 or more units have been associated.
3. In many species the presence of numerous dot-like chromosomes, such as could be centromere-units, similar to the parts of the other types of chromosome.

Text-fig. 3 illustrates these chromosome types.

The parallel separation of chromatids, the independent passage to the poles of fragments, are both explicable by the hypothesis of the 'diffuse' centromere. However, comparison with other organisms allows one to bring forward the strong evidence in favour of polycentry available in the case of *Luzula*. The comparison will first be completed:

1. All species show parallel separation of chromatids.

2. Metaphase chromosomes are few and large or smaller and more numerous (*Spirogyra, Luzula, Coccids*?).
3. The shape of large metaphase chromosomes is sinuous, with sticky matrix (*Spirogyra, Luzula, Coccids*); that of small chromosomes is rounded rod or dot (*Spirogyra, Luzula, Carex*).
4. Fragments behave as whole chromosomes in the spindle (*Luzula, Coccids, Spirogyra*—still to be confirmed in experiment).
5. Discontinuous staining of prophase chromosomes (*Spirogyra*; *Luzula*, verbal communication from Lima de Faria).
6. Certain chromosome types appear to be associations of two or three units at metaphase, and are found in the same complement as single small units each with localized centromere (*Spirogyra*; Text-fig. 1).
7. In *Luzula* only we find the most interesting evidence of all regarding the nature of the large chromosomes, Nordenskiöld (1951) having hybridized species with large and small chromosomes and shown that at meiosis small chromosomes pair with parts of the large ones.

Whether or not one accepts the view of Nordenskiöld that the small chromosomes have resulted from fission of the large ones, surely in this case one can hardly doubt that the large chromosomes possess a number of separate centromeric structures, each corresponding to one found in a small chromosome.

It seems to me that the chromosomes of all these organisms are in essentials structurally alike, and that in view of what is known about them, to retain the hypothesis of the 'diffuse' centromere when the much more credible hypothesis of polycentry fits all the facts yet known is unnecessary, although one can imagine circumstances in which there might be a basis for using either term. One may perhaps go farther and state that so far as the evidence goes we have in *Spirogyra*, at least, a new kind of chromosome which appears to be composed of several kinds of material:

(*a*) That composing the deeply staining blocks. This is associated with centromeric activity.
(*b*) That composing the intervening regions. It exists only, or mainly, in those species with large chromosomes—these are compound.
(*c*) That composing the N.O. region. This has no centromeric activity.
(*d*) That composing the 'matrix'. This is 'nucleolar substance' (see Godward, 1953). Presumably the staining blocks and the N.O. regions should be regarded as heterochromatic. The peculiarities of spiralization are perhaps consequent on the unusual structure.

It is a temptation, in regard to *Spirogyra*, to put forward the almost obvious theory on the evolution of the localized centromere which suggests itself. In this unique genus where there are so many different types of chromosome, some (Text-fig. 1 and Pl. V, Figs. 7, 8) plainly suggesting that small units each with a centromere may have become added together (Text-fig. 3), others that a polycentric structure was followed by the predominating influence of one centromere, parallel separation of chromatids could become changed to separation of the usual type. It is true that some of the small chromosomes which are not compound are nevertheless big enough to show a localized centromere, and others with a sub-terminal centromere have a long trailing region; the N.O. region also trails behind the large chromosome which is presumed to be otherwise composed of small units. It is clear that not all parts of the large chromosomes, and not all the medium-sized chromosomes, are polycentric. It is of interest that if an attempt to distinguish homologous chromosomes is made, it is the big compound chromosomes which show evidence of homology and would correspond with the chromosome types of higher plants; such would, however, be expected. It is presumably to be assumed that long chromosomes can in any case only have arisen as a result of the coherence of small units, whatever the value of the evidence presented for one genus.

ACKNOWLEDGEMENT

The author is indebted to Dr. Darlington and Mr. L. La Cour for discussion of certain points (in 1949), and to Professor I. Manton and Mr. Brian Clarke for Pl. V, Figs. 2 and 6.

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EXPLANATION OF PLATES

Illustrating M. B. E. Godward's paper on 'The "Diffuse" Centromere of Polycentric Chromosomes in *Spirogyra*'

PLATE V

FIG. 1. Mid-prophase, *S. sub-echinata*. The two N.O. chromosomes are on the left, each showing seven stained blocks with intervening material, the two N,O. regions still surrounded by part of the dissolving nucleolar material. On the right, the two other chromosomes each showing five stained blocks and intervening material. There is a suggestion of somatic pairing between these two. Four chromosomes in two pairs of homologues. (×2,500.)

FIG. 2. Mid prophase, *S. sub-echinata*. Ultra-violet photograph. The same features are visible as in Fig. 1. Both satellites are not in focus—see the transparency. (×2,500.)

FIG. 3. Early prophase, *S. sub-echinata*. Twenty of the twenty-four stained blocks visible at this stage are shown in the photograph. The intervening material is not visible at this stage. (×2,000.)

FIG. 4. Prophase stage intermediate between those of Figs. 1 and 3, part of Text-fig. 2, *S. sub-echinata*. The less staining material intervening between the stained blocks is seen, showing two chromatids and suggesting that they are coiled round one another. (×3,300.)

FIG. 5. Early prophase of *S. crassa*, showing the stained blocks, arranged linearly. (×930.)

FIG. 6. The metaphase chromosome of *S. sub-echinata*. Photograph by ultra-violet light (3,750 A). This is the N.O. chromosome and the pale appearance of the N.O. region is well seen. Neither chromatids nor coiling are visible. (×3,000.)

FIGS. 7 and 8. Side view of the metaphase of *S. brittannica*, showing median and sub-terminal localized centromeres, also small dot-like chromosomes. (×3,300.)

PLATE VI

FIG. 1. *S. triformis*, sister anaphase plates; compare the shapes of the chromatids with Fig. 3, below. The stained blocks are beginning to reappear. The shapes of sister chromatids are nearly identical. (×2,600.)

FIGS. 2 and 3. The metaphase (N.O.) chromosome of *S. triformis*, Fig. 2 under-printed from the negative, to show the internal coil and Fig. 3 fully printed, showing the 'matrix'. The N.O. region is at the top. (×4,000.)

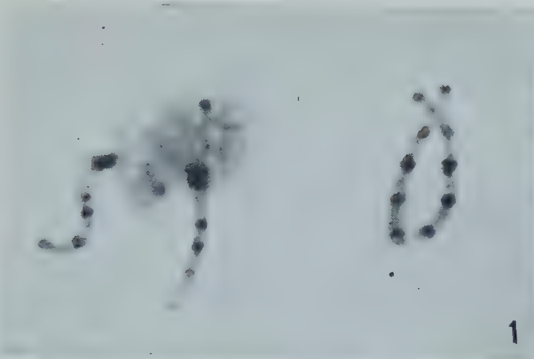
FIG. 4. *S. crassa*, sister anaphase plates, showing the nearly identical shapes of sister chromatids (see also Godward, 1953). Two of the N.O. regions are clearly seen. (×2,000.)

FIG. 5. *S. crassa*, metaphase plate, showing the sinuous shape of the chromosomes and the fact that there is no sign of a localized centromere; chromatids are not seen. (×1,300.)

FIG. 6. *S. crassa*, parallel separation of chromatids, and indication of 'compound' structure of the chromatid. (×4,000.)

FIG. 7. *S. crassa*, as Fig. 6, the whole plate in side view. (×1,300.)

FIG. 8. *S. sub-echinata*, parallel separation of chromatids. (×2,000.)



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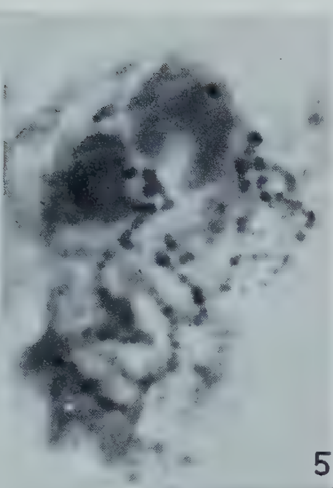

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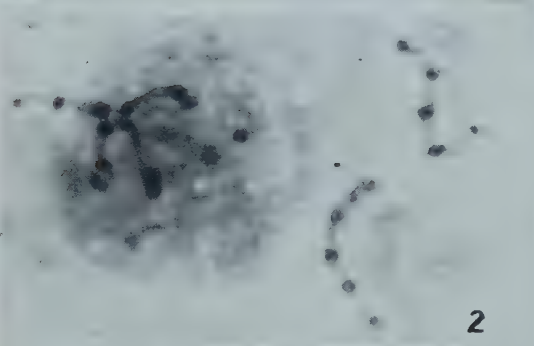

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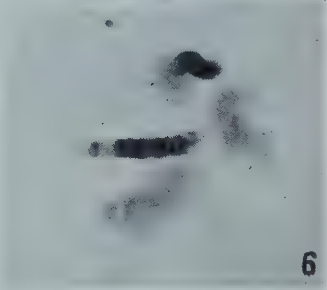

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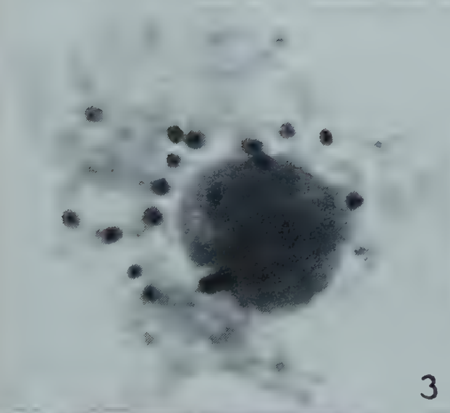

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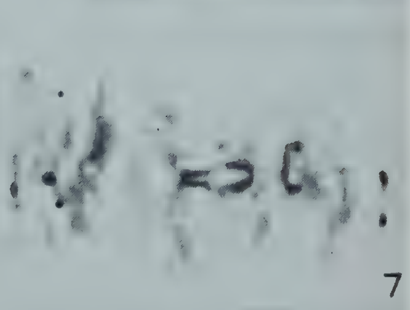

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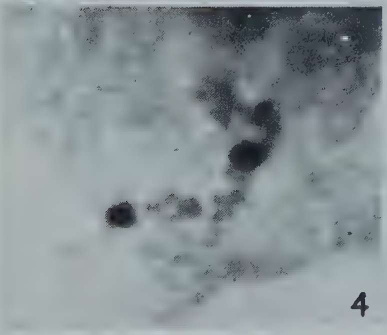

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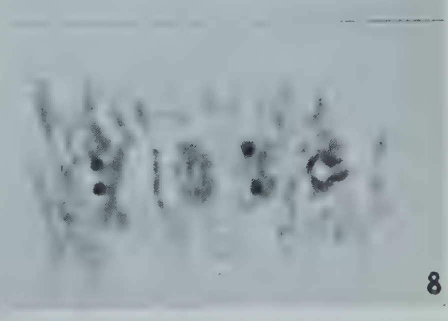

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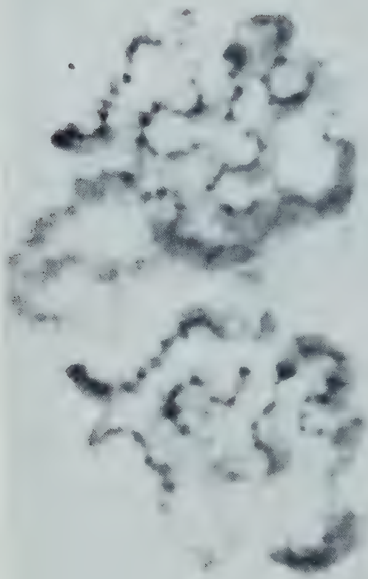
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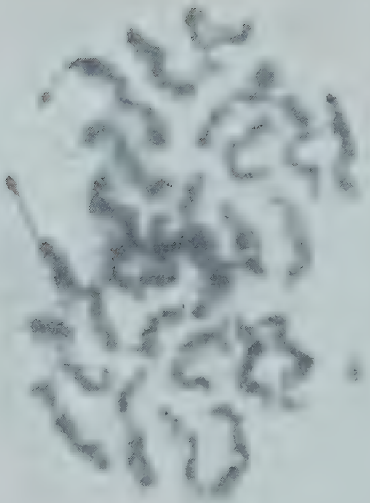
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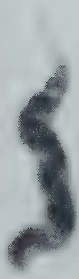
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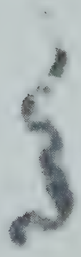
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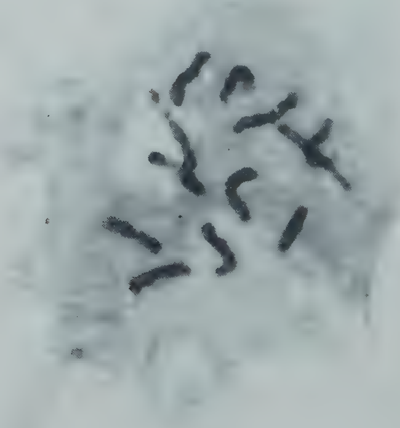
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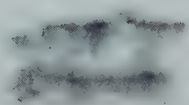
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M. B. E. GODWARD

The Use of the Infra-red 'Image Converter' Viewing Apparatus in the Study of the Effects of Localized Wounds on the Growth of Etiolated *Avena* Seedlings

BY

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With Plate VII and three Figures in the Text

ABSTRACT

Experiments are described in which etiolated *Avena* seedlings were briefly exposed to infra-red radiation and observed with an 'image converter' apparatus for the purpose of wounding them accurately at predetermined places. Data on the effects of wounding on the subsequent growth of the seedlings in darkness are presented and discussed in terms of the auxin theory of growth regulation.

INTRODUCTION

VAN OVERBEEK (1935, 1936) has attributed mesocotyl growth in the etiolated *Avena* seedling to auxin reaching it from the coleoptile tip. Experiments carried out to test this hypothesis indicated that the meristematic complex at the node might exercise some control over mesocotyl extension for, when the node was removed, mesocotyl growth was considerably reduced (Mer, 1951). Severing the node from the seedling involved the simultaneous removal of the coleoptile and so excluded the possibility of estimating the influence of the node, if any, on the behaviour of the coleoptile.

This paper describes experiments in which different regions of etiolated seedlings (particularly the node, the basal part of the coleoptile and plumule, or the coleoptile tip) were wounded in an attempt to determine their influence on the further development of the plants. The method of wounding employed, described below, was designed to ensure that all the organs of the seedling remained in contact after treatment.

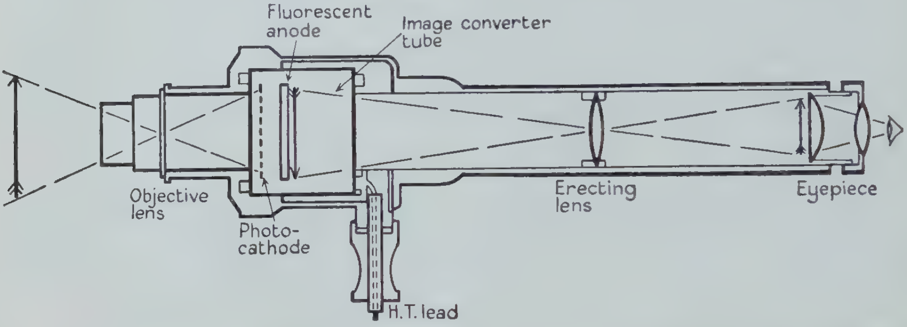
MATERIALS AND METHODS

Avena sativa var. 'Victory' was used for all experiments. The seed was planted as previously described (Mer, in press) and the plants, 6 sets with 57 in each, were grown in a light-tight container in an incubator operated at 24° C. ± 0·2° C. Over each set of plants an inverted litre-beaker was placed, lined with damp blotting-paper to maintain saturation, and a stream of air saturated with water-vapour swept continuously over the seedlings at a rate of 10 litres/hour.

[Annals of Botany, N.S. Vol. XVIII, No. 70, April, 1954.]

It was essential to manipulate and wound the plants in complete darkness because exposure to light reduces the length of the mesocotyl. Wounding similarly reduces its length and so, had the plants been illuminated in order to wound them precisely at a predetermined place, the effects of illumination and wounding would have been confounded.

Many attempts at wounding the node in darkness were made, with scant success, and it became apparent that, to perform the operations successfully, the seedling would have to be observed by some means which did not subsequently alter its growth in darkness. It was known that objects exposed to infra-red radiation may be seen by means of an 'image converter' (Pratt, 1948), and, as Goodwin (1941) has shown that the growth of etiolated oat seedlings is unaffected by brief exposures to infra-red, the technique of observing in this way has been employed in these experiments.



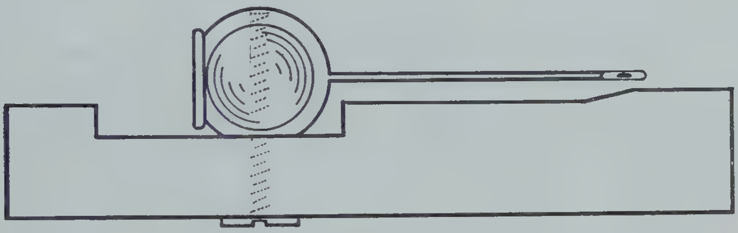
TEXT-FIG. 1. 'Image converter' viewing apparatus. Redrawn from Electronic Engineering, 1948. For description see text.

The image converter apparatus is an electronic device which transforms infra-red to visible radiation. A diagram of the 'viewer' is shown in Text-fig. 1. The crucial part of the apparatus is the image converter tube, a cylindrical glass cell some 2 in. in diameter and 1·5 in. long, which has mounted within it two electrodes, a cathode, and an anode. The cathode is a flat disc composed of a silver-oxide-caesium mixture, while the anode is a screen maintained at a potential of 2,000 V. D.C. by means of a power-pack run from a 6-V. accumulator. Infra-red radiation reflected from the object is focused by an objective upon the cathode. This, in consequence, emits electrons which impinge upon the anode, which fluoresces under their impact. The visible image so formed is then viewed by an erecting eyepiece. The brightness of the image depends upon the density of the electron stream impinging on the anode, and this in turn depends on the reflectivity of the object for the radiation. Consequently, variations in the surface texture of the object are transformed on the fluorescent anode into corresponding variations of brightness.

The differences in structure between the mesocotyl, a solid stem, and the coleoptile, a hollow cylinder with leaves enclosed, are sufficient to cause variations in the reflection of infra-red from the surface of the seedling, and the node and the insertion of the leaves upon it may thus be located with reasonable accuracy.

A hypodermic needle (0·44 mm. in diameter) was used for wounding the plants, chosen not only because of its sharp cutting-edge but also because it could easily be held by the expanded base which normally serves for attaching it to the syringe barrel. In preliminary experiments, in which the seedling was held in one hand and the needle in the other, accurate wounding proved to be difficult because of the very shallow depth of focus of the 'viewer' objective. The slightest movement of the hands put the image out of focus and the plants, instead of being pierced, were almost invariably wounded to one side. With such treatment the seedlings frequently broke at the wound.

To overcome these difficulties the needle was rigidly fixed employing the basal part for this purpose. It was partially filed away at one side then drilled, tapped, and screwed down to a short brass bar (Text-fig. 2). To ensure that the plants were pierced rather than cut laterally a gap of about 0·5 mm. was left between the needle-tip and the upper surface of the mount by cutting a recess as shown in the figure.



TEXT-FIG. 2. Method of mounting the hypodermic needle used for wounding the plants.

It was further found that contact with the mount prevented the free movement of the plant on to the needle. This friction, coupled with a slight resistance to penetration, resulted in many broken plants. The upper surface of the mount was consequently sloped under the needle-tip as shown so that, once pierced, the seedling was out of contact with it and frictional resistance was eliminated. To reduce reflections from it the mount was coated with matt-black lacquer. It was clamped firmly in a suitable position with the tip distal, and the 'viewer' was also clamped, conveniently inclined, after adjustment to bring the needle-tip into sharp focus.

Infra-red radiation was obtained from a 240-V. 100-watt lamp (under-run at 110 V.) and a Wratten filter (587/1) which, when used with a low-temperature source, transmits the IR frequencies but absorbs the whole of the visible spectrum. The density of the filter at 840 mμ. (as measured on a Uvispek) is 0·7, while at 780 mμ. it is 3·3 and is here increasing very rapidly with decrease of frequency. The lamp was connected in series with a foot 'pressel-switch' so that, with the other apparatus fixed, both hands were left free to manipulate the seedlings.

The technique finally adopted for irradiating and wounding 3-day-old plants was as follows: in darkness a set of seedlings was removed from the light-tight container, which was closed again, and placed behind a screen to shield the plants from stray radiation. HT was switched on and the seedling for treatment was placed in position across the mount just beyond the needle. The foot-switch was operated, the appropriate part of the plant applied to the needle-tip and pulled down on to it. The switch was then released, wounding completed, and the seedling replaced behind the screen in darkness. When all the plants in the set had been treated they were replaced in the container and, at the same time, the next set was removed for treatment. Each irradiated plant was thus exposed for 3–6 seconds only; controls were similarly exposed for 10 seconds but without wounding. After treatment the plants grew for a further 4 days in darkness and so were 7 days old when measured.

EXPERIMENTAL RESULTS

1. *The effect of irradiation*

To establish that exposure to infra-red radiation did not reduce mesocotyl growth, control experiments were carried out in which approximately half the number of plants were irradiated for 10 seconds, as already described, while the remainder were not exposed. The results of two experiments recorded in Table I show that the mean lengths of control and irradiated plants did not differ significantly. In experiment 2 the difference between mesocotyl mean lengths approached significance because the control plants were unusually short.

TABLE I

Lengths (mm.) of Control and Irradiated Plants. Replicate numbers in brackets. Variability is the standard error

	Irradiated		Not irradiated	
	Mesocotyls.	Coleoptiles.	Mesocotyls.	Coleoptiles.
Expt. 1	62·2±0·61	72·9±0·53 (143)	62·5±0·57	72·2±0·55 (168)
Expt. 2	61·1±0·62	71·7±0·59 (162)	59·7±0·67	71·1±0·71 (140)

2. *The interaction between irradiation and wounding*

The object of these experiments, one of which is recorded in Tables II and III, was to determine whether or not interaction took place between irradiation and wounding.

The treatments were: (*a*) controls, (*b*) mesocotyl wounded somewhat below the node, and (*c*) coleoptile wounded near the tip in each of two series—irradiated and not irradiated, forming a 3×2 factorial arrangement. The plants in each set were randomly sorted into 6 groups, each with approximately 9 plants, and one treatment was applied to each group. Thus for the whole experiment there were 36 groups.

The difficulty of wounding the node in darkness has already been mentioned. It was quite simple to wound the coleoptile tip in the dark, but rather than attempt to treat the node likewise and have finally an assortment of plants treated differently, with some wounded through the node, some below it, and some above it through the base of the coleoptile, it was decided to wound the mesocotyl well below the node; in practice some 4–6 mm. It

TABLE II

Expt. 3. The Effects of Irradiation, Wounding, and their Interaction. Lengths (mm.) of plants derived from 282 observations. Analysis of variance computed using mean values

		Controls.	Mesocotyl wounded.	Coleoptile wounded.
Irradiated	Mesocotyls	59·7	42·4	58·5
	Coleoptiles	69·0	49·9	57·8
Controls	Mesocotyls	58·7	43·5	58·2
	Coleoptiles	68·7	48·5	56·0

Analysis of variance

		Mesocotyls		Coleoptiles	
	DF.	Mean sq.	F. ratio.	Mean sq.	F. ratio.
Irradiation	1	0·24	n.s.	95·45	n.s.
Wounding	2	7886·97	155·56*	9176·25	62·30*
Interaction	2	26·74	n.s.	14·84	n.s.
Error	246	50·70	—	147·30	—

* *P* 0·01 requires 6·76.

TABLE III

Expt. 3. The Effect of Irradiation, Wounding, and their Interaction. Lengths of plants in mm.; 24 replicates per treatment obtained by random selection from 282 original observations

		Controls.	Mesocotyl wounded.	Coleoptile wounded.
Irradiated	Mesocotyls	59·4	42·0	59·8
	Coleoptiles	70·4	51·0	56·6
Controls	Mesocotyls	59·2	42·9	58·3
	Coleoptiles	68·3	48·0	54·3

Analysis of variance

		Mesocotyls		Coleoptiles	
	DF.	Mean sq.	F. ratio.	Mean sq.	F. ratio.
Irradiation	1	3·07	n.s.	222·51	n.s.
Wounding	2	4461·76	88·61*	4965·78	36·28*
Interaction	2	16·39	n.s.	2·86	n.s.
Error	108	50·35	—	136·84	—

* *P* 0·01 requires 6·90.

was possible to perform this operation in darkness although, inevitably, the wound was not always accurately placed.

Due to variation in germination and inadvertent breakage in handling, the final number of seedlings per treatment varied from set to set. The appropriate statistical treatment for sub-groups with unequal numbers is detailed in Snedecor (1948, section 11.12), but the computations involved are laborious. As the numbers per treatment were not very different, an analysis using the mean lengths per group was carried out, assuming that they were estimated with equal accuracy. The error variance was obtained independently from the within groups sums of squares. It was consequently based upon a single plant, whereas the mean squares relating to irradiation, wounding, &c., calculated from the mean lengths referred to an average of 7·83 plants per group. The mean squares were made mutually comparable and were put on a single plant basis by multiplying each of the treatment mean squares by 7·83. The mean lengths of the groups of plants and the analysis of variance are recorded in Table II.

TABLE IV

The Effects of Wounding the Node or Plumule on the Growth of Seedlings

	Expt. 4	
	Mesocotyls.	Coleoptiles.
(*a*) 3-day-old seedlings	35·5±0·86	12·2±0·11 (49)
7-day-old plants:		
(*b*) controls (not irradiated)	61·3±0·90	67·4±1·05 (48)
(*c*) irradiated controls	60·7±0·98	66·4±1·05 (44)
(*d*) ,, and coleoptile tip wounded	59·1±0·82	57·9±1·22 (48)
(*e*) ,, ,, node wounded	43·9±0·88	49·0±2·30 (45)
(*f*1) ,, ,, plumule wounded	52·7±0·91	50·7±1·96 (43)
(*f*2) ,, ,, plumule removed	50·3±1·26	61·6±3·81 (8)

	Expt. 5	
	Mesocotyls.	Coleoptiles.
(*a*)	27·1±0·75	11·1±0·10 (46)
(*b*)	60·0±0·76	71·7±0·84 (49)
(*c*)	59·1±0·86	72·2±1·08 (45)
(*d*)	58·9±1·03	61·1±1·40 (33)
(*e*)	37·1±1·02	44·3±2·83 (41)
(*f*1)	49·8±0·96	51·3±1·95 (47)
(*f*2)	44·8±1·89	63·0±5·37 (6)

A second computation was undertaken in which the plants per group were randomly reduced to the smallest number found in any one set, namely 4. The measurements and analysis of variance are given in Table III. Because this procedure entailed a wastage of half the available information (144 observations used from a total of 282) this second analysis is regarded as purely confirmatory.

In effect, both analyses of the data yield identical results, and for this reason it was considered unnecessary to undertake the fitting of constants as described by Snedecor.

To confine attention to the factors under consideration, they alone are quoted in the analyses of variance. It is apparent that the effect of wounding was highly significant, while the effects of irradiation and its interaction with wounding were, on the contrary, quite negligible.

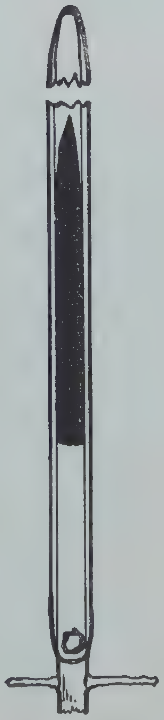
The measurements show that when the coleoptile tip was wounded the length of the coleoptile was reduced, while that of the mesocotyl was hardly affected. The mesocotyl wound, however, caused a reduction in the length of both organs.

3. *Main experiments*

In these experiments, which could not be arranged factorially, the intention was to investigate the influence of the node and of the plumule on the growth of the plants. The plumule was wounded by inserting the needle just above the node through the basal part of the coleoptile. The plants were randomly distributed among the treatments shown in Table IV which, as in previous experiments, were replicated within each of the sets of seedlings.

The measurements of the plants in treatments *b*, *c*, and *d* conform with those in the corresponding treatments in expt. 3. It will be seen that wounding the node causes a greater reduction in the lengths of both mesocotyl and coleoptile than does any other treatment. The effect is comparable with that produced by wounding the mesocotyl below the node in expt. 3. The plants in treatment *f* have been subdivided into groups because when they were examined a few were found with the plumule severed from the mesocotyl and carried up by the coleoptile as it continued to grow (as shown in Text-fig. 3), while in the remainder the plumule, although wounded, had continued to grow.

The measurements of the plants in these two treatments show that the mesocotyls were shorter than those in which the coleoptile tip had been wounded (treatment *d*). The coleoptiles also showed a reduction in growth, but less so in *f*2 than in *f*1.



TEXT-FIG. 3. Diagram of the coleoptile showing its appearance when the plumule had been removed and the cut-off leaves had been carried upwards by its further growth.

One further difference in the behaviour of the plants may be noted. When the node had been inactivated by piercing (treatment *e*) the nodal roots failed to grow. In treatment *f*, although the wound was made quite close to the node, these roots grew normally.

4. *The effect of wounding the mesocotyl superficially*

In the experiments described so far, particular care had been taken to pierce the plants when wounding them, and it has been shown that when the mesocotyl was so treated, either through the node or below it, the growth of the coleoptile was greatly reduced. It may be recalled that the mesocotyl has a single, central vascular bundle which branches in the vicinity of the node, so that the nodal meristem is traversed by the traces supplying the coleoptile and stem apex with water and nutrients. The two wounding treatments mentioned above effectively interrupted the conducting system. It was therefore thought desirable to wound the mesocotyl superficially and so determine the effect of a treatment which did not interfere with the vascular bundles.

TABLE V

Expt. 6. Lengths (mm.) of Plants wounded superficially at the Node or below it

	Mesocotyls.	Coleoptiles.
Irradiated controls	64·6±0·51	67·8±0·41 (262)
,, and wounded at the node	50·7±0·24	61·8±0·26 (253)
,, ,, below the node	56·7±0·49	63·3±0·51 (251)

To do this the gap between the needle-tip and the mount was increased so that the flank of the plant was wounded. Three experiments were carried out, each of which gave a similar result. The data were therefore combined and the mean lengths are recorded in Table V.

The effect of wounding was here much less pronounced than in the previous experiments. The superficial nodal wound caused a greater reduction in the growth of both mesocotyl and coleoptile than did a similar wound below the node. The majority of the wounded plants showed a sharp kink at the wound (with the wound on the concave surface) while a few had coiled as shown in Pl. I, both mesocotyl and coleoptilar tissue being involved in the curvature. The presence of the roots at the node, usually developed on the unwounded flank, indicated that the nodal meristem had continued to function even when the node had been wounded.

DISCUSSION

The technique of observing seedlings by the use of infra-red radiation during the performance of delicate operations has proved to be entirely satisfactory. There is no occasion to discuss here the effects of longer exposures to infra-red radiation, as direct experiments to test the reaction of the plants to the brief exposures actually used showed that there was no effect on their subsequent growth in darkness.

The method of investigation employed depended upon wounding the plants and the effects of such wounds are unavoidable. In this respect the investigation differs little from others designed to test the effects of growth-promoting substances, which entail, for instance, the removal of the coleoptile tip as in both the standard *Avena* (Went, 1928) and straight growth methods (Scheer, 1937), or the isolation of sleeves of coleoptile tissue as in the method originally introduced by Bonner (1933). In the 'Pea' test (Went, 1934) an even more extensive mutilation is involved. Assay methods in which the test plants are not wounded are, however, available, notably the root method (Moevus, 1949) and the parthenocarpic development of tomato ovaries (Luckwill, 1948).

TABLE VI

Percentage Reduction in the Growth of Plants due to wounding

	Mesocotyls.	Coleoptiles.
1. Coleoptile tip pierced	2	17
2. Node pierced	32	33
3. Node wounded superficially	22	9
4. Mesocotyl pierced below the node . . .	27	29
5. Mesocotyl superficially wounded below the node	12	6

The effect of a wound, wherever situated, may lead to the production of a wound hormone and the possibility cannot be overlooked of differential effects of wounding depending on the site of the wound.

The decapitation of a coleoptile will have a three-fold effect: first, the removal of the source of auxin; second, the regeneration of a physiological tip and secondary production of auxin; and third, the production of a possible wound hormone. Piercing the coleoptile, on the other hand, will leave intact the source of hormone in the tip, but auxin transport or that of its precursor may be radically affected, particularly if the vascular system is interrupted. The coleoptile may, however, be wounded so as to leave the vascular bundles intact, in which case only the effect of a wound hormone, local secondary auxin production, or local interruption of transport in the parenchymatous tissue would result. Experiments designed to assess the influence of the vascular bundles on the growth of the coleoptile are now in progress.

Excluding for the moment those treatments involving the plumule, the reduction in the growth of the mesocotyl and coleoptile as a result of wounding may be summarized as in Table VI.

These summarized data make possible certain deductions regarding the effects (*a*) of wounding, (*b*) of injuring the node, and (*c*) of interrupting the vascular system.

(*a*) It will be seen by comparing entries 1, 3, and 5 that the effect of wounding on the coleoptile was a maximum when the tip was wounded and that the effect diminished as the site of the wound receded from the tip. This is in accord with the current view that the tip, in which auxin synthesis takes place, controls the growth of the coleoptile. Similarly with the mesocotyl, wounding the node had the largest effect; wounds at other places had less effect on its growth, that at the coleoptile tip occasioning the least response. It may therefore be inferred that the node functions with respect to the mesocotyl as the tip does for the coleoptile. Van Overbeek *et al.* (1945) have found in the sugar-cane that the nodes contain more extractable auxin than do the internodes, but the data of Van Overbeek (1938) on the auxin concentrations in the mesocotyl of *Avena* are considered to be questionable on the grounds that the plants were exposed to light during handling.

(*b*) The superficial injury to the node affected mesocotyl growth to a greater extent than did a corresponding wound below the node. The very much greater effect of piercing the node on mesocotyl growth may be attributed to the combined effects of the nodal injury and the interruption of the vascular system. If the superficial mesocotyl wound, which reduced growth by 12 per cent. (entry 5 in Table VI), may be regarded as a mere wound effect, then the greater effect of the superficial nodal wound (22 per cent.) may be regarded as direct injury to the node and the difference (10 per cent.) would be an estimate of this effect. It might then be expected that adding this estimate of the nodal effect to that of the pierced mesocotyl wound would equal the effect of piercing the node. The calculated value (37 per cent.) is somewhat higher than the actual value of 32 per cent., which may indicate that the effects are not strictly additive as has been assumed.

The effect of the node on the growth of the coleoptile may be estimated in the same way. The difference between entries 5 and 3 (3 per cent.) would measure the effect of the node on the coleoptile, and this added to entry 4, making 32 per cent., agrees well with 33 per cent., the observed effect of the pierced nodal wound on coleoptile growth.

(*c*) The vascular system evidently plays an important part in determining the growth of the seedling. Over and above the 22 per cent. reduction in mesocotyl growth due to the superficial nodal wound there was a further reduction of 10 per cent. when the node was pierced and the vascular system disrupted (the difference between entries 3 and 2). This may be compared with a 15 per cent. extra reduction in growth due to interrupting the vascular bundle in the mesocotyl itself (entries 5 and 4). The greater effect of piercing the vascular bundle in the mesocotyl might be due to the fact that the single strand here was broken and so caused a total disruption of the conducting path, whereas when the node was pierced some of the minor strands in this region might have escaped injury.

The conclusion to be drawn from this analysis is that the activity of the node has a preponderant effect on the growth of the mesocotyl but, in addition, the continuity of the vascular system is important for the growth of the seedling as a whole. So far as wounding is concerned, this also has an effect which diminishes as the wound recedes from the active centres controlling growth.

The interruption of the vascular system will interfere with the movement of water, of auxin and precursors, and of the substrates for growth. The connexion of the coleoptilar vascular bundles with tropic phenomena was appreciated, according to Boysen-Jensen (1910), by Rothert in 1896, and Laibach and Kornmann (1933) have shown that larger curvatures of the coleoptile resulted when auxin was applied externally over the bundle than when it was applied to the coleoptile between the bundles. They further showed by using coleoptiles suitably cut away at the top that, when a hormone-containing block was placed upon the vascular bundle, larger curvatures were obtained than when it was placed on the broader side of the coleoptile away from the vascular bundle. Nevertheless they concluded that the role of the bundle in relation to hormone transport remained undecided.

The parts of the seedling proximal and distal to the wound differ from each other with respect to these three factors. Considering the wound at the node, the mesocotyl proximal to it was in contact with the endosperm and roots and hence with the reserve of carbohydrates, auxin precursors, and water-supply (many of the mesocotyls of the wounded plants appeared translucent as if waterlogged), and its reduced growth would be accounted for either by an effect on the meristem, in that the number of potentially elongating cells was reduced, or by the absence of growth-promoting substances normally derived from the coleoptile and stem apex.

It is at present impossible to discriminate between these alternatives.

The coleoptile distal to the wound was not in direct vascular connexion with the roots, but as the plants were grown in a saturated atmosphere there should not have been any considerable water strain. As the tip was uninjured the coleoptile would presumably have adequate supplies of auxin. There is also a reserve of starch in the tip cells so that carbohydrates would be available. On the basis of this argument it would seem that an absence of precursor was the factor limiting the growth of the coleoptile.

In spite of the saturated atmosphere, however, lack of water seems more probably the cause of the reduced growth. Previous work has shown that the seedlings are very sensitive to the ambient humidity level, for instance a fall of 10 per cent. in relative humidity resulted in a 30 per cent. decrease in the growth of the plants (Mer, in press). This point will need to be tested by experiments in which the distal parts of the plant are in some way supplied with water.

The influence of the plumule on the growth of the seedling may now briefly be considered.

There is undoubtedly some mechanism co-ordinating the growth of the coleoptile and plumule for they react in the same way, for instance, to a brief exposure to light. This retards mesocotyl growth but stimulates that of the coleoptile and plumule and they grow in step, the leaves filling the coleoptile, until they emerge when the coleoptile stops elongating. The data in Table IV (treatments *f*1 and *f*2) provide some information on the effect of the plumule. They show that when it was removed the coleoptiles were longer and the mesocotyls shorter than when it was only wounded. The effect on the coleoptile was more pronounced than that on the mesocotyl and it would appear, therefore, that the effect of the plumule was to retard the growth of the coleoptile. It has been mentioned that wounding the plumule in these experiments necessitated wounding the base of the coleoptile as well. The recorded reductions in growth are consequently due to the combined effects of these wounds. To estimate the effect of the plumule alone further information is required on the response of the seedling to wounding the base of the coleoptile only. The difference between the two sets of data would then indicate the effect of the plumule. The necessary experiments have been performed, and the measurements are summarized, together with the data of treatments *f*1 and *f*2, in Table VII.

TABLE VII

Percentage Reduction in Growth due to wounding the Plumule

	Mesocotyls.	Coleoptiles.
Base of coleoptile wounded	6	6
Base of coleoptile and plumule wounded	15	27
Base of coleoptile wounded and plumule removed	21	11

The results indicate that the plumule does affect the growth of seedling, but because of the inadequacy of the data on the effect of removing the plumule it is considered undesirable to carry the analysis further at present.

In conclusion the evidence presented in this paper points to the node as being the centre controlling the growth of the mesocotyl and the coleoptile tip must be regarded as secondary in this respect.

SUMMARY

The effects of wounding either the coleoptile tip, the coleoptilar node, or stem apex on the further growth of etiolated oat seedlings has been studied.

The wounds were accurately placed by exposing the plants briefly to infra-red radiation and observing them by means of an 'image converter'.

It is shown that the brief exposure to infra-red radiation does not affect the subsequent growth of the plants in darkness.

The greatest effect on mesocotyl growth occurs when the node is wounded. A superficial wound in this region reduces growth by 22 per cent.: a further reduction of 10 per cent. takes place when the vascular system is interrupted.

The growth of the coleoptile is also reduced when the node is wounded; the greater part of the reduction is due to the interruption of the vascular bundles.

Wounding the plumule also reduces the growth of the seedlings, but the data are considered to be too meagre to warrant drawing firm conclusions.

The results indicate that the node is the centre controlling mesocotyl growth.

ACKNOWLEDGEMENTS

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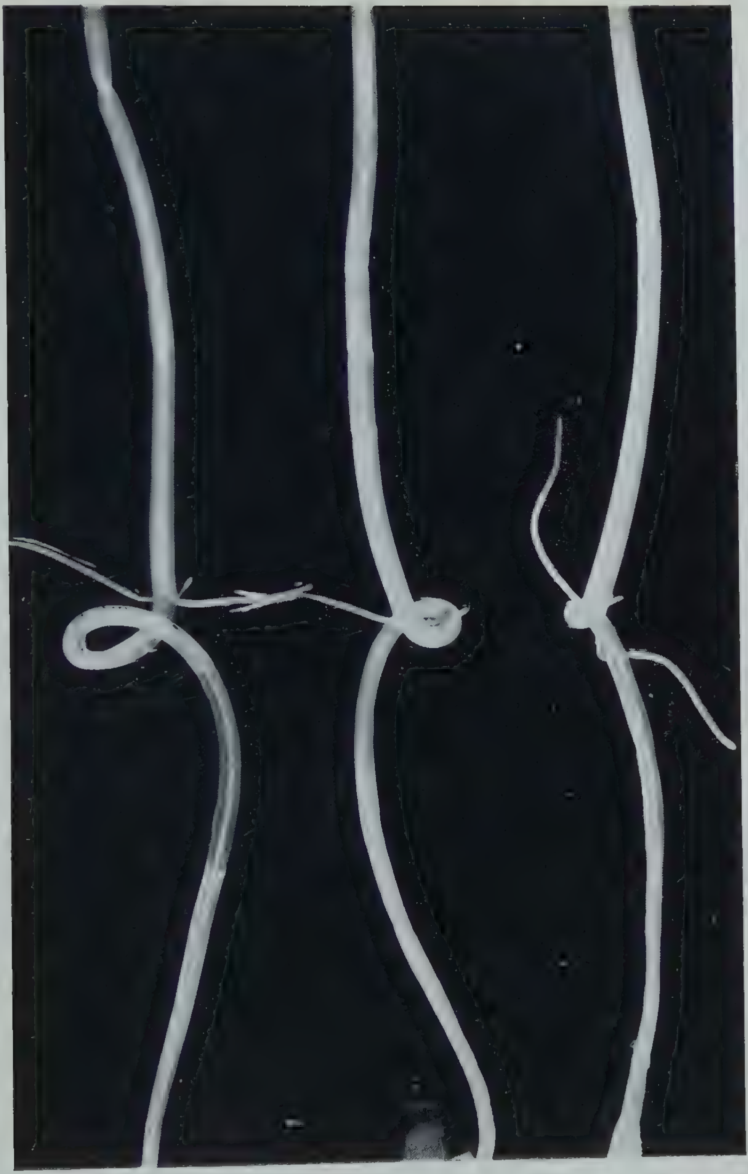
EXPLANATION OF PLATE VII

Illustrating C. L. Mer's paper on 'The Use of the Infra-red "Image Converter" Viewing Apparatus in the Study of the Effects of Localized Wounds on the Growth of Etiolated *Avena* Seedlings'.

Three coiled seedlings. Distorted growth resulting from a superficial flank wound.

The left-hand seedling was wounded below the node, whose position is indicated by the rootlets, and mesocotyl tissue only contributes to the formation of the loop. The middle and right-hand seedlings were both wounded at the node and in the middle one the lower half of the loop is formed by the coleoptile.



C. L. MER

Experimental and Analytical Studies of Pteridophytes

XXV. Morphogenesis in *Selaginella Willdenovii* Baker

II. *Angle-meristems and Angle-shoots*[1]

BY

F. CUSICK

With Plate VIII and seven Figures in the Text

ABSTRACT

The structure of the 'angle-meristems' of *Selaginella Willdenovii* is described. An account is given of the formation of dorsiventral shoots from these meristems and some experiments on causal aspects of dorsiventrality in *Selaginella* are reported and discussed.

PAGE
1. INTRODUCTION 171
2. MATERIALS AND METHODS 172
3. TERMINOLOGY 172
4. ANGLE-MERISTEMS 172
5. DORSAL AND VENTRAL ANGLE-SHOOTS 174
6. THE DIFFERENTIATION OF VASCULAR TISSUE 176
7. EXPERIMENTS ON ANGLE-SHOOTS 176
8. DISCUSSION 179
9. SUMMARY 180
ACKNOWLEDGEMENTS 181
LITERATURE CITED 181

1. INTRODUCTION

IN the genus *Selaginella* meristems occur at points of branching of the axis and may be termed 'angle-meristems'. They characteristically develop as rhizophores but their development as shoots is not uncommon in whole plants and seems to occur without exception when dormant angle-meristems are isolated from the axial apex. The formation of rhizophores from such isolated meristems can, however, be induced by treatment with β-indole-acetic acid (Williams, 1937). Thus dormant angle-meristems, with their potentiality for growth either as rhizophores or as shoots, are useful materials for the study of morphogenesis. Unlike axial and branch apices, which cannot be exposed without drastic dissection, they can be operated on directly. In an earlier paper (Cusick, 1953), the origin of dorsal and ventral angle-meristems at a

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[1] The work reported here was carried out in the Department of Cryptogamic Botany, Manchester University, and submitted for publication from the Botany Department, Aberdeen University.

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bifurcation of the axial apex was considered. The present account begins with the dormant meristems and goes on to describe the formation from them of shoots; the effects of some experimental treatments of angle-meristems are then described and discussed.

2. MATERIALS AND METHODS

Angle-meristems were induced to form shoots by the method of Behrens (1897) in which portions of the axis were removed from greenhouse plants and laid on moist peat in a germinator. The subsequent growth of the undeveloped angle-meristems into shoots was studied by regular inspection with dissecting microscope, and by examination of serial sections of fixed materials. Experimental treatments were made immediately after isolation of the twigs.

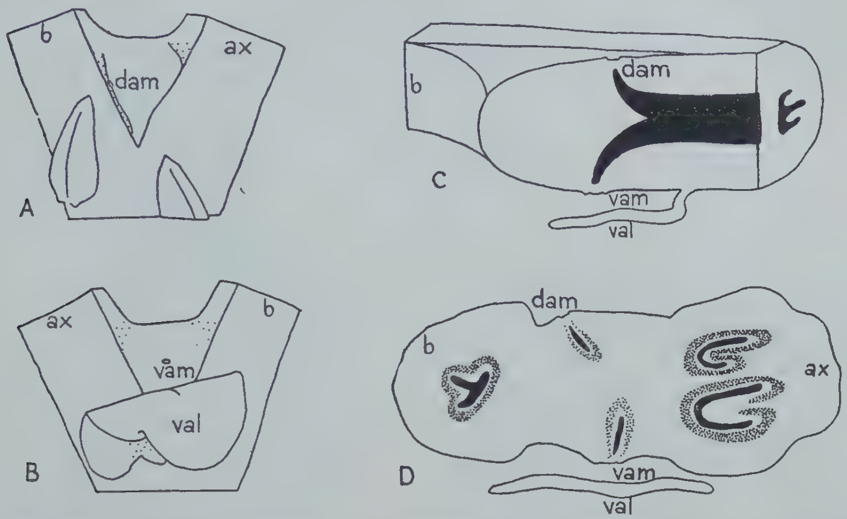
3. TERMINOLOGY

It will be recalled that there are differences between the growth-patterns of the two shanks that are formed by the dichotomy of the shoot apex. These have led to the recognition of one as *axis* and the other as *branch* (Cusick, 1953). The shoots coming from dormant angle-meristems are here termed angle-shoots. They are, in a broad sense, branches of the axis; but they grow like the axis itself, not like the branch as already defined. Because of this, reference will be made to the axis of the angle-shoot and to that of the parent shoot.

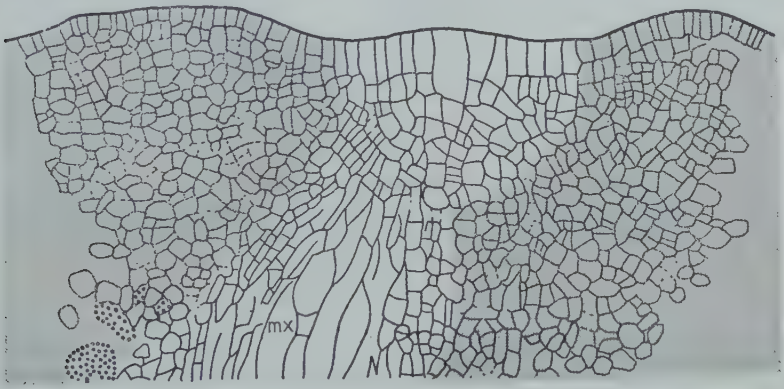
The structure and growth of any part of the shoot system of *S. Willdenovii* may, of course, be described by reference to three planes or co-ordinate axes: *longitudinal* (i.e. proximal-distal); *dorsiventral* (passing vertically through both dorsal and ventral surfaces); and *lateral* (in which branching takes place).

4. ANGLE-MERISTEMS

In *S. Willdenovii*, a dorsal and a ventral angle-meristem occur at each axial bifurcation (Text-fig. 1). They are formed superficially in the apical region between axis and branch and continue as potentially meristematic areas when the surrounding tissue matures into parenchyma. At first, they comprise only a small number of cells; as these segment, a central *apex* of large cuboid cells and a peripheral *rim* of small-celled meristematic tissue are differentiated (Text-figs. 2 and 3, A). In surface view, both angle-meristems are for a time circular; later they become elliptical. The greater dimension of the ventral meristem is lateral on the parent shoot, that of the dorsal meristem is parallel to the adjacent branch. On the ventral surface of the shoot, the ventral angle-meristem occupies a median position on the soft tissue between the shanks of the bifurcation; on the dorsal surface the dorsal meristem is much nearer to the branch than to the axis (Text-fig. 1). Angle-meristems sometimes remain dormant for a long time, only a fraction of a millimetre high and showing no macroscopic growth. Commonly, however, ventral meristems grow out as rhizophores when about six dichotomies behind the axial apex; dorsal meristems develop much later, usually as shoots. The precocity of ventral over dorsal angle-shoots is also shown in experimental materials. In twigs isolated with both angle-meristems undeveloped, the ventral meristem grows out first, whatever the orientation of the twig, and in the presence or absence of light; if rhizophores are induced by the application of β-indole-acetic acid, the ventral rhizophore is the first to develop. The greater reactivity of the ventral angle-meristem at this stage, which is also expressed in its larger size, is evidently independent of external factors (Cusick, 1949).



TEXT-FIG. 1. Axial bifurcations: A, in dorsal and B, in ventral surface view (× 5); C, in longitudinal and D, in transverse section (× 10). *ax*, axis; *b*, branch; *dam*, dorsal angle-meristem; *vam*, ventral angle-meristem; *val*, ventral angle-leaf; xylem, solid black; lacunae, stippled.

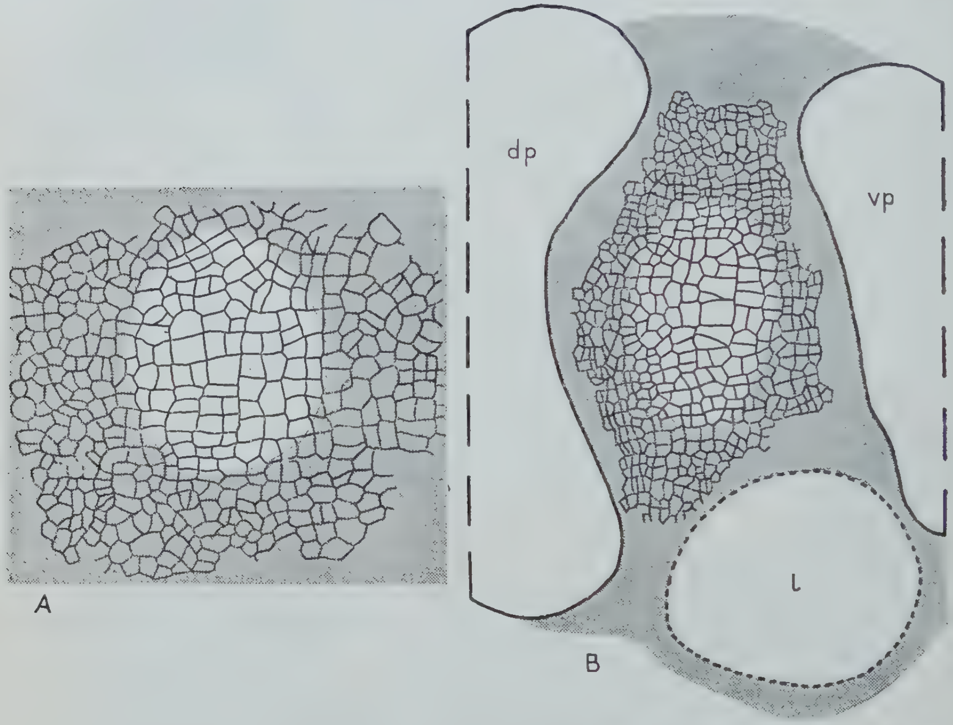


TEXT-FIG. 2. Vertical section through a dormant ventral angle-meristem (× 200). Apex and stelar tissue unstippled; rim (dorsal and ventral sectors shown) and subjacent tissues finely stippled; lacunae coarsely stippled; *mx*, metaxylem.

The internal anatomy of the angle-meristem is shown in Text-fig. 2.

5. DORSAL AND VENTRAL ANGLE-SHOOTS

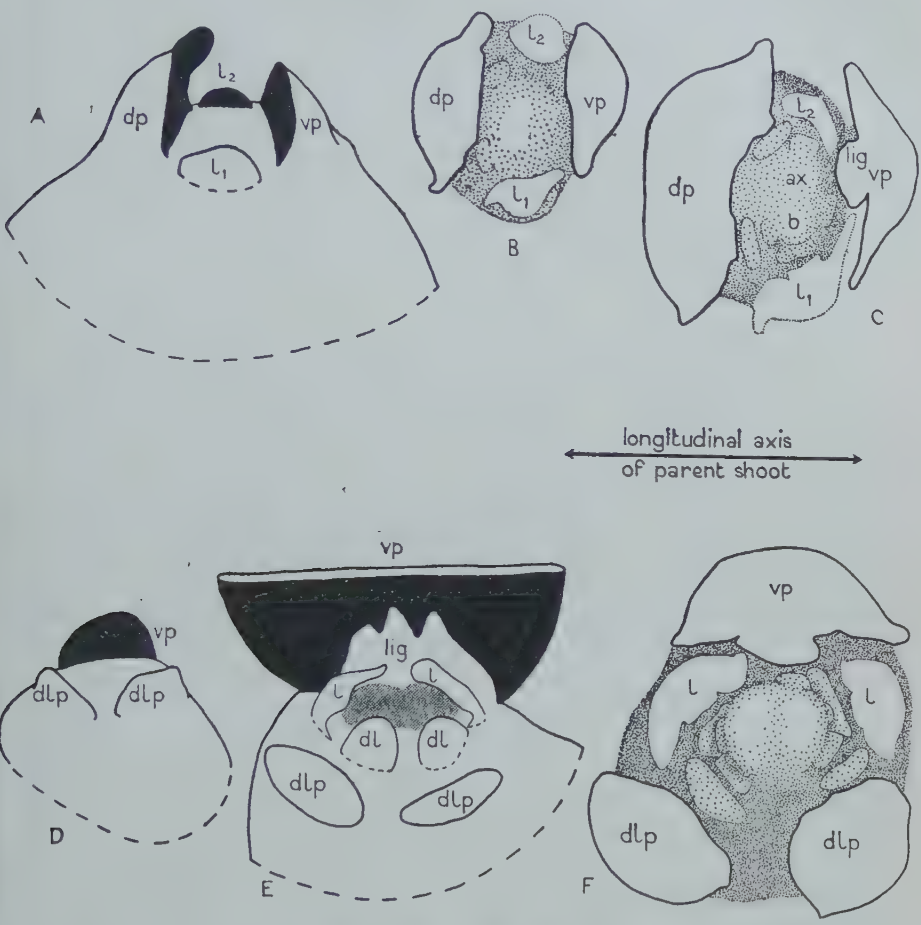
Conspicuous changes occur in an angle-meristem within a few days from its isolation from the axial apex. The bilateral symmetry of the whole meristem becomes more marked. The longer dimension is the lateral axis of the new shoot and the longer arcs of the rim give rise to prophylls. It is convenient to name the prophylls according to the dorsiventrality of the *new* (angle-) shoot. In ventral angle-shoots there is one dorsal and one ventral prophyll, and the dorsal surface is distal on the parent shoot (Pl. VIII). In dorsal angle-shoots, a single ventral prophyll forms from the long arc of the rim that is against the *branch* of the parent shoot, and two dorsilateral prophylls form from the opposite arc. There is, therefore, almost a right angle difference in the orientations of dorsal and ventral angle-shoots to the parent shoot. Prophylls resemble ordinary leaves except in their more massive origin; occasionally they show two ligules, side by side (dorsal prophyll in Text-fig. 4, c). Within the prophylls the formation of leaves now takes place acropetally on the flanks of the apex. A lateral leaf develops between the ventral and the



TEXT-FIG. 3. A, surface view of a dormant ventral angle-meristem: rim, surrounding central apex, stippled; the section becomes internal to the right of (dorsal to) the apex. B, surface view of young ventral angle-shoot: *dp*, *vp*, cut bases of dorsal and ventral prophylls; *l*, primordium of first lateral leaf; cells of apical region unstippled, subjacent regions stippled. (Both × 200.)

dorsal (or a dorsilateral) prophyll and is followed by a dorsilateral leaf; this by the next lateral leaf.

During prophyll formation the cells along the summit of the apex form a



TEXT-FIG. 4. A, B, C, ventral angle-shoots. A, showing prophylls and first lateral leaves from the side; B (in plan) shows primordia of dorsilateral leaves between the lateral leaves and the dorsal prophyll; C shows further lateral and dorsilateral leaf primordia, also the primordium of a ventral angle-leaf (below the bifurcated apex, on the ventral side). D, E (side views), F (in plan), comparable developments in dorsal angle-shoots (the main difference is in orientation to the parent shoot). In F, a ventral angle-leaf is distal to the ventral prophyll. *dp*, *vp*, *dlp*, dorsal, ventral, and dorsilateral prophylls (or their cut bases); l, l_1, l_2, lateral leaves; *dl*, dorsilateral leaf; *lig*, ligule. (All × 52.)

band of wedge-shaped apical cells—the cell-pattern that characterizes the apex of the adult shoot of *S. Willdenovii*. After a few days, increased growth is noticeable at the two foci of the elliptical apex and shows that branching has taken place. A ventral angle-leaf now develops on the ventral surface of the shoot, below the two shanks. These changes are illustrated in Text-figs.

3, B and 4. Dorsal and ventral angle-meristems are later differentiated where the inter-apical groove meets the dorsal and ventral surfaces of the angle-shoot.

In the ventral angle-shoot the gap between dorsal and ventral prophylls is greater on the side that is adjacent to the axis of the parent shoot, and the leaf series on this side makes its appearance before the opposite series. But when the apex bifurcates, it is the shank towards the parental branch that continues the axis of the angle-shoot; the shank adjacent to the parental axis becomes the first branch. In dorsal angle-shoots the first lateral leaves appear more or less simultaneously. When the shoot bifurcates the shank that is distal on the parental shoot continues as the axis, the proximal shank forming the first branch. Fries (1911) has described differences of orientation between dorsal and ventral angle-shoots in *S. Martensii.*

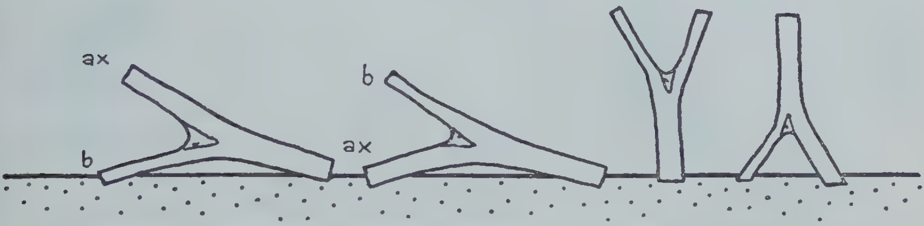
6. THE DIFFERENTIATION OF VASCULAR TISSUE

The vascular supply to the angle-meristems separates from the axial stele at a point slightly distal to the separation of the stele of the branch. Here it consists of a dorsiventral strap of xylem, surrounded by phloem and pericycle and partially isolated from the cortex by lacunae. Near the angle-meristems, dorsal and ventral halves of the strap separate; each ends in prestelar tissue immediately below the apex of the corresponding angle-meristem (Text-figs. 1 and 2; also Wardlaw, 1944, Plate III, Fig. 5). When an angle-meristem grows out as a shoot, new vascular tissue differentiates in continuity with that already formed; its disposition, however, differs markedly from the single-banded structure of the angle-meristem phase. The xylem band now has one or more salients from either face, though there is considerable diversity in detail at this stage. As the shoot grows the configuration of the new vascular tissue changes; the xylem salients become separated from the main band, giving rise first to a tri-xylic, then to a 'tri-stelic' state (Wardlaw, 1925). The vascular bands are in the lateral plane of the angle-shoot and, as has been seen, this in dorsal angle-shoots is approximately longitudinal, in ventral angle-shoots transverse, on the parental shoot. At the base of the ventral angle-shoot there is a change of 90 degrees in the orientation of the stele, corresponding to the active growth of the young angle-shoot across the long axis of the parental shoot. The transition can be seen in angle-shoots that are forming their first lateral leaves; the salients become more massive and the original main band is reduced. The vascular traces of prophylls and leaves are small in proportion to the size of the vascular straps.

7. EXPERIMENTS ON ANGLE-SHOOTS

(*a*) *The effects of gravity and of light*

The constancy of orientation to the parental shoot which angle-shoots display is seen in a greenhouse culture, where plants are growing in many directions; it is maintained on upright and on prostrate shoots, and on inverted twigs. Dorsal angle-shoots growing out at successive bifurcations alternately face opposite ways, as the branches alternate to either side of the axis. It is therefore unlikely that the directions of light and gravity affect appreciably the plane of dorsiventrality. The experiments of Pfeffer, Goebel, and Fries (see Cusick, 1953) support this view. The present writer has fixed isolated twigs bearing dormant dorsal angle-meristems in four vertical positions (Text-fig. 5); the dorsiventrality and orientation of the resulting angle-shoots (13 in all) were normal.



TEXT-FIG. 5. Orientation of dorsal angle-meristems to gravity. *ax*, axis; *b*, branch. (Natural size.)

(*b*) *The partial isolation of the angle-meristem from the parental shoot*

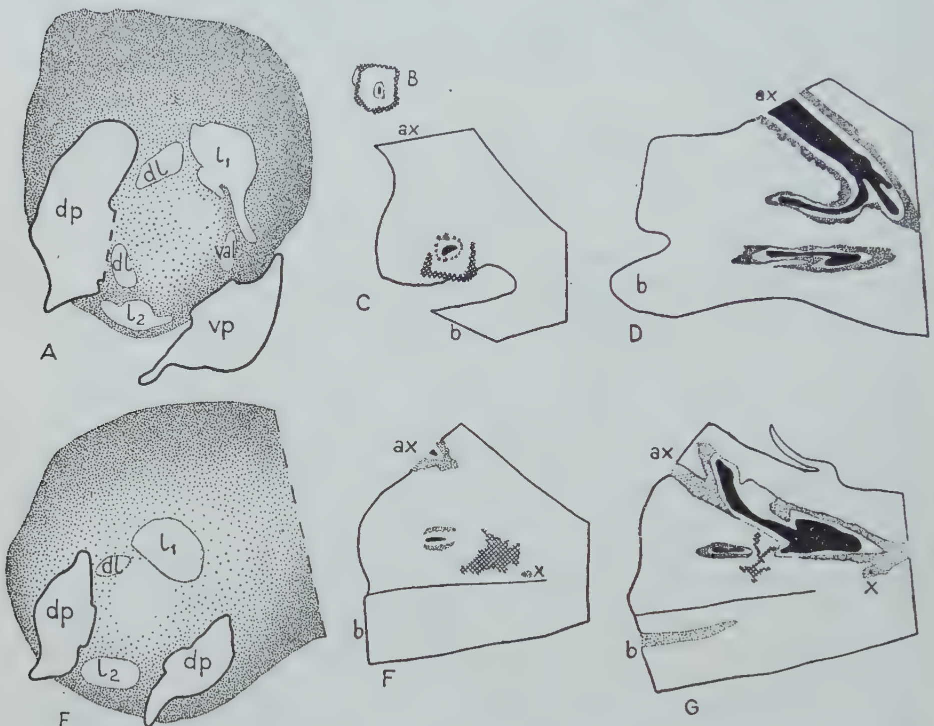
As dorsiventrality and orientation are not determined by external factors, this experiment was designed to show whether or not the remains of the parental shoot induce morphogenetic effects. Dormant ventral angle-meristems on isolated twigs were separated from the surrounding tissue by the removal of thin slices of tissue outside the rim; each angle-meristem, therefore, was situated on a plug of tissue that had only basal continuity with the parent twig. Nine angle-shoots, comprising five square and four triangular isolations, developed successfully from the thirteen meristems used. In all of them, prophylls were formed in their normal positions; the subsequent appearance of lateral leaves and (in those that were more advanced at fixation) dorsilateral and ventral angle-leaves was also normal (Text-fig. 6, A–D). Vascular differentiation was not noticeably affected by either the isolation itself or the shape of the plug.

A second experiment was carried out in which a single panel of tissue was removed from six isolated twigs, a short distance behind their dormant ventral angle-meristems, so that the vascular strand of each meristem was severed. Normal angle-shoots developed (Text-fig. 6, E–G). (In one shoot, an additional leaf occurred between the ventral prophyll and the first lateral leaf; this abnormality has occasionally been seen in untreated shoots.)

These experiments indicate that the factors underlying dorsiventrality are, at this stage, present within the angle-meristem itself.

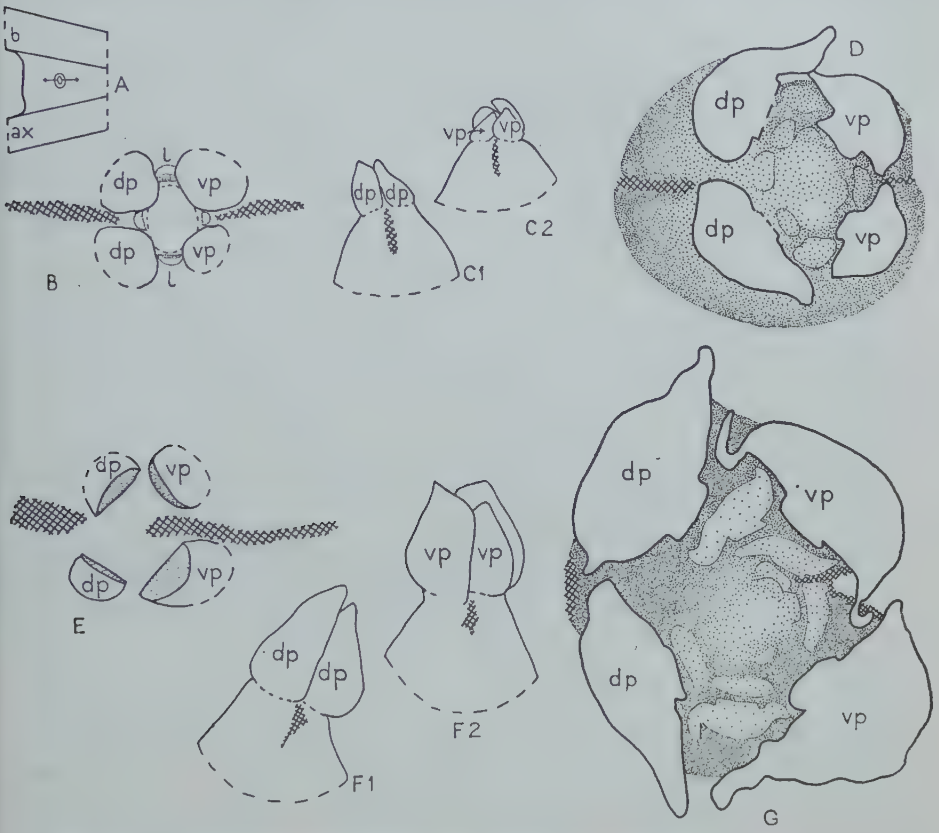
(*c*) *Radial incisions through the presumptive prophylls*

In this experiment an attempt was made to change the orientation of the prophylls; it was considered that if such changes could be induced, interesting changes in the apical regions might follow. Four ventral angle-meristems were used. Immediately after the isolation of the twigs a median radial cut was made through the presumptive position of each prophyll. Within a week, two prophylls, one on each side of the experimental cut, were forming from each presumptive area. The two separated halves of a prophyllar growth-centre are evidently still more capable of growth than are the lateral sectors of the rim, and they reorganize as whole leaves. At the end of the second week all the young angle-shoots had bifurcated.



Text-fig. 6. Partially isolated angle-meristems. A, construction from serial sections of distal regions (× 52); B–D, sections at lower levels showing extent of isolation (× 10). E, distal region of an angle-shoot (× 52); F, G, sections at lower levels showing severance of vascular strand (× 10). Incisions cross-hatched; xylem black; lacunae finely stippled; *x*, trace of ventral angle-leaf; other lettering as above.

Although the orientation and dorsiventrality of the angle-shoots was not changed by the experimental treatment, interesting disturbances of morphogenetic pattern occurred within the rim, in the neighbourhood of the incisions. In two angle-shoots, a leaf developed above the dorsal cut, in the approximate position of a dorsal angle-leaf. One of these is illustrated in Text-fig 7, B–D. The other was more complex, the 'dorsal angle-leaf' being continuous basally with a prophyll and a lateral leaf. Both these shoots also developed an additional leaf between the ventral incision and the true ventral angle-leaf. In the third shoot, an extra leaf developed to one side of the ventral incision, above the prophylls and below the true ventral angle-leaf. In the fourth shoot (Text-fig. 7, E–G), a leaf developed on each side of the ventral cut, where this penetrated into the apex; subsequently, a leaf series developed above these



Text-fig. 7. Radial incisions through the presumptive prophylls. A, diagram of experiment; arrows mark the cuts. B, an angle-meristem after 1 week (× 36); two dorsal and two ventral prophylls, normal lateral leaf primordia, and a leaf primordium distal to each incision are visible. C 1, dorsal and C 2, ventral views (× 12), and D, construction from serial sections (× 52), after 2 weeks; a normal ventral angle-leaf is developing distally to the induced ventral leaf. E, another meristem after one week (× 36). F 1, dorsal and F 2, ventral views (× 12), and G, a construction from serial sections (× 52), after 2 weeks; a ventral angle-leaf is developing on the ventral slope of the bifurcating apex, further explanation in Text. *dp*, *vp*, dorsal and ventral prophylls; *l*, lateral leaf; *b* and *ax*, branch and axis of parent shoot, orientated as in the first apex (B–D)—in E–G their positions were reversed; wounds cross-hatched.

two leaves, and the lateral axis of the shoot came to be oblique above the normally developed first lateral leaves.

8. DISCUSSION

The surgical treatments described have failed to alter the positions of the prophylls and the first lateral leaves. It seems likely, therefore, that the growth-centres for these organs are already present in the dormant ventral angle-meristem. If this is so, growth regulating substances in the intact plant (and β-indole-acetic acid applied artificially to isolated twigs) must, in certain concentrations, allow the development of the apical but not the appendicular growth-centres; a rhizophore, leafless and with a superficial radial symmetry, is the result. In the absence of these growth-regulating substances (brought about by the removal of the axial apex), apical and appendicular growth-centres show macroscopic growth within a few days, and form a leafy shoot-system. These observations might suggest that, in ontogeny, the angle-meristem is basically an embryonic shoot and that rhizophore formation involves a secondary change of growth-pattern. It is possible, however, that some features of rhizophoric pattern are also to be found in the dormant meristem.

The formation of extra leaves in close proximity to radial incisions through the prophylls is of considerable interest. These leaves have developed both distally and laterally to the wounds. They have always been orientated to the apex (not to the cuts), and they have not been closely associated with the small wound callus. Induction by wound hormones, though not disproved, is hardly supported by these considerations. The tissue that has given rise to the leaves has not been isolated from the apex or from other leaves. Wardlaw (1948) has demonstrated, in *Dryopteris*, the formation of leaves in positions of minimal stress; the incisions now under discussion probably reduced tensile stress in their neighbourhood. It is also possible that the wound destroys inhibiting concentrations of growth-regulating substances. If the mechanism of the induction of these extra leaves were known, the normal absence of leaves on the dorsal and ventral surfaces of shoots of *S. Willdenovii* might be more easily understood.

Differences in orientation between dorsal and ventral angle-shoots, as yet unexplained, are probably related to the different growth-patterns of the dorsal and ventral surfaces of the dichotomizing axial apex.

9. SUMMARY

1. The dormant angle-meristem of *Selaginella Willdenovii* consists of an *apex* of large cuboid cells surrounded by a small-celled *rim*. When an angle-shoot develops, prophylls are formed from the rim; distally, the apex forms a dorsiventral shoot system of normal structure.

2. Differences in orientation to the parent shoot and in prophyll arrangement exist between dorsal and ventral angle-shoots, but for either class morphogenetic pattern can be predicted.

3. Morphogenetic pattern is unaffected by orientation to light and gravity, or by continuity with the surrounding tissues.

4. Experimental evidence from ventral angle-meristems suggests that growth-centres are already present in the positions of prophylls and lateral leaves at a time when the angle-meristem is still capable of rhizophoric development. In the presence of the parental axial apex or of applied β-indole-

acetic acid, sub-apical but not apical growth-centres are inhibited and a rhizophore is formed.

5. Leaves have been induced on the normally untenanted dorsal and ventral surfaces of the shoot by means of vertical incisions.

ACKNOWLEDGEMENTS

The author thanks Professor C. W. Wardlaw for his interest and help, and Mr. Ernest Ashby for his photographs of angle-meristems and shoots.

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EXPLANATION OF PLATE VIII

Illustrating Dr. F. Cusick's paper on Angle-meristems and Angle-shoots in *Selaginella Willdenovii* Baker.

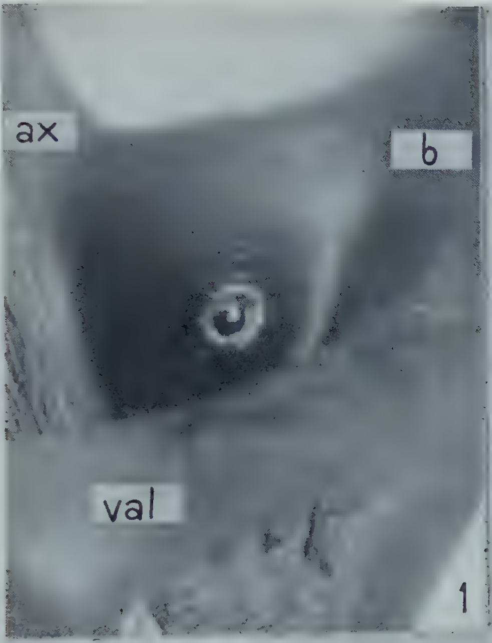
FIG. 1. A dormant ventral angle-meristem, immediately after separation from the parent shoot system, showing apex and rim.

FIG. 2. The same meristem, 9 days after separation, showing dorsal and ventral prophylls and lateral leaves.

FIG. 3. The same meristem, 14 days after separation; the prophylls now conceal the apex.

FIG. 4. Another ventral angle-meristem, 18 days after separation from the parent shoot system; lateral view, showing dorsal and ventral prophylls and a lateral leaf.

ax, axis and *b*, branch of parent shoot; *val*, ventral angle-leaf. All photographs × 20.



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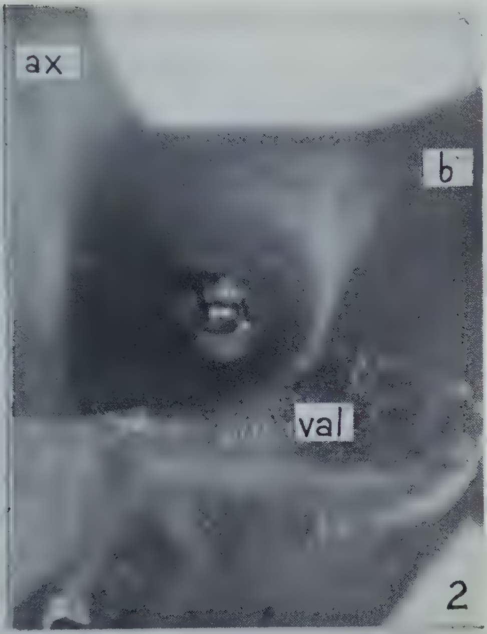

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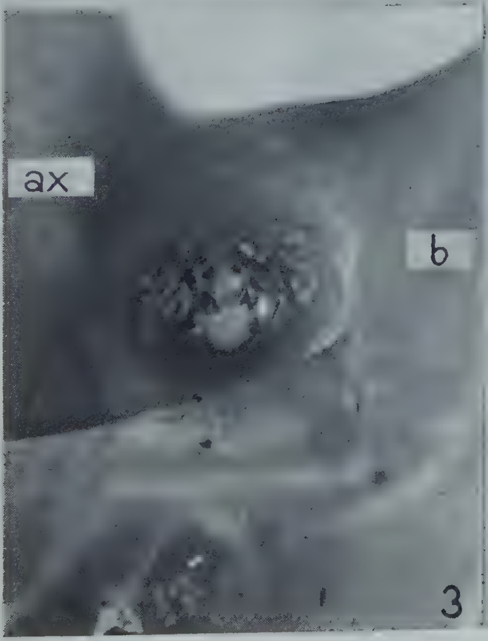

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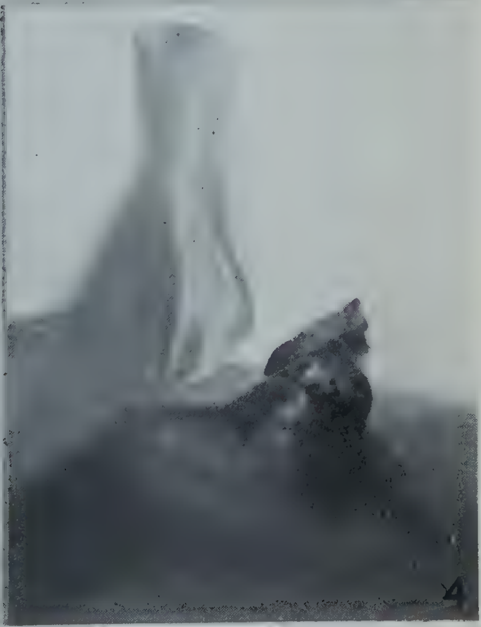

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F. CUSICK

Studies in the Bangioideae

III. The Life-history of *Porphyra umbilicalis* (L.) Kütz. var. *laciniata* (Lightf.) J. Ag.[1]

A. *The Conchocelis-phase in culture*

BY

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With Plates IX–XII and five Figures in the Text

ABSTRACT

As the first step in an investigation of the life-history of *Porphyra umbilicalis* (L.) Kütz. var. *laciniata* (Lightf.) J. Ag., spores which have originated as a result of the repeated division of the mother-cells have been germinated. When grown on glass their method of germination and growth agrees with that described by previous investigators, but when germinated on sterile shell the germ-tubes penetrate the shell and develop into growths identical with *Conchocelis rosea* Batters. This 'species', therefore, is a phase in the life-history of *P. umbilicalis* var. *laciniata* and not an autonomous species. The development of the '*Conchocelis*'-phase in culture is described and also the formation of fertile cell-rows and 'plantlets'. Until the significance of these structures is understood and a cytological investigation completed, the relationship of the intertidal leafy *Porphyra*-thallus to the filamentous, shell-inhabiting, deep-water *Conchocelis* cannot be expressed in the terms usually employed to denote the various phases of the life-histories of algae.

PAGE
1. INTRODUCTION . . . 184
2. CRITICAL SURVEY OF LITERATURE REGARDING THE LIFE-HISTORY OF *PORPHYRA* . 185
3. MATERIAL AND METHODS . . . 190
4. EXPERIMENTS TO DEMONSTRATE THE DEVELOPMENT OF *CONCHOCELIS ROSEA* BATTERS FROM SPORES OF *PORPHYRA UMBILICALIS* (L.) KÜTZ. VAR. *LACINIATA* (LIGHTF.) J. AG. . . . 192
5. THE *CONCHOCELIS*-PHASE UNDER CONDITIONS OF CULTURE . . . 197
   (*a*) Vegetative development . . . 197
   (*b*) Fertile cell-rows . . . 200
   (*c*) 'Plantlets' . . . 202
6. DISCUSSION . . . 206
7. SUMMARY . . . 208
ACKNOWLEDGEMENTS . . . 209
LITERATURE CITED . . . 209

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[1] The plant used for this investigation is similar to that figured by Kylin (1944, Taf. 1, fig. 2) under the name *Porphyra umbilicalis* (L.) Kütz. f. *laciniata* (Lightf.) J. Ag. At a later date (1945) Kylin gave this form specific rank, but subsequently (1949) united it again with *P. umbilicalis* (L.) Kütz. The present writer is not prepared at this stage to produce evidence supporting either of these alternatives, and hence for the time being continues the use of the above nomenclature without, however, acknowledging the identity of this material with *Porphyra laciniata* of C. Ag. (1824) or *Ulva laciniata* of Lightfoot (1777).

[Annals of Botany, N.S. Vol. XVIII, No. 70, April, 1954.]

1. INTRODUCTION

As the facts accumulate, it is becoming increasingly obvious that the life-histories of algae show much greater variation within the limits of a class, or even smaller groupings than was considered to be the case two or three decades ago. For example, it is no longer possible to divide the Rhodophyceae on the basis of their life-histories into the two sections defined by Svedelius (1915) as haplo- and diplobiontic. This situation suggests that there is need for fresh concepts of the significance of the life-history. Such concepts can only be built satisfactorily on the basis of information obtained from complete investigations of life-histories and experimental work to determine the fundamental factors controlling them. There are difficulties inherent in this type of investigation so that it may not be possible to obtain all the desired information in a particular case, but the attempt should be made and nothing is gained scientifically (and in fact much may be lost) by a failure to recognize the existence of gaps in the information. Even apparently complete and logical accounts based on a minimum of observations may be misleading, and it is possible that further investigation of 'classical' species might well result in additions to or modifications of existing 'classical' accounts.

In an investigation into the life-history of an alga the aim should be to obtain a series of observations of the development of the thallus from the germinating spore and then of the development of the reproductive organs on the mature thallus. In cases where both sexual and asexual spores are formed the germination of both types of spore should be followed, and the type of reproductive organ formed on the resulting thallus ascertained. In addition, information about the nuclear cycle is essential, such as the place of fertilization and meiosis as well as the chromosome complement of the nuclei of thalli bearing various types of reproductive organs. In order to assess the effect of environmental factors on the formation and development of the various types of reproductive organs, and the consequent effect on the life-history, observations on the species not only in its natural habitat at all times of the year but also under controlled conditions in culture are necessary. Information regarding the occurrence of the various types of reproductive organs throughout the area in which the species occurs is of significance in estimating to what degree a sequence of events established as occurring in one locality is obligatory throughout the whole area of its distribution. Observations on such points as vegetative propagation and perennation are also relevant.

Porphyra umbilicalis (L.) Kütz. var. *laciniata* (Lightf.) J. Ag. was selected for investigation along the lines indicated, for the following reasons: It occurs abundantly over a wide area of the Northern Hemisphere and the existing literature indicates that the spores germinate readily, so that it seemed legitimate to assume it could be cultured. Secondly, a critical consideration of the results and conclusions put forward by previous workers with regard to the reproduction and the life-history of *Porphyra* showed clearly that there existed considerable need for further investigation of many points under these headings. Lastly, the scanty and somewhat conflicting statements about nuclear details showed a renewed and extended cytological investigation to be necessary.

Such an investigation is very lengthy, and this paper records only one section of the work, that dealing with the germination of spores produced by the leafy *Porphyra* thallus. These spores are formed by repeated division of the mother-cell, the first wall being parallel to the surface of the thallus. It has been found that such spores give rise to a filamentous shell-inhabiting phase, identical with growths previously known from the sea under the name of *Conchocelis rosea* Batters (1892). *C. rosea* can no longer be considered an autonomous species, therefore. The experimental evidence demonstrating the connexion between *C. rosea* and *P. umbilicalis* is given in the first section, and this is followed by an account of the development in culture of the *Conchocelis*-phase as well as the formation upon it of fertile cell-rows and 'plantlets'.

2. CRITICAL SURVEY OF LITERATURE REGARDING THE LIFE-HISTORY OF *PORPHYRA*

The genus *Porphyra* belongs to the Bangiaceae, the only family of the Bangiales for which sexual reproduction is known and which shows any advance on the filamentous thallus. A full historical survey of investigations into the reproduction of the Bangioideae is being published elsewhere, and so a critical consideration of the results and conclusions put forward by various workers relating to *Porphyra* only is given here.

Although the genus *Porphyra* is one of the most common of marine algae of the temperate regions, and various species have been the subject of frequent investigations, an attempt to state even in bare outline the sequence of the somatic and nuclear phases of the life-history shows how little has been definitely established. The earlier investigators, particularly Janczewski (1873), made careful observations of the vegetative structure and some aspects of the reproductive processes, and later Berthold (1882) gave an account of the reproductive processes, which account has been accepted in the main by subsequent algologists. Later investigators, with the exception of Dangeard (1927, 1931) and Kunieda (1939), have narrowed their work to attempts to close the gap in the life-history between the germination of the spores considered carpospores by Berthold and the reappearance of the leafy thallus. This has produced at least four theories regarding the unknown part of the life-history.

In order to ascertain how much of the life-history has been definitely established, as well as the extent and value of the existing evidence regarding it, the critical events in the life-history will now be considered one by one. It should be remembered that some species, either throughout their areas of distribution or in parts of them, are completely absent for certain months of the year.

Considering first the development of the leafy thallus from the spore, both Yendo (1919) and Kunieda (1939) have described the early stages in its development from unicellular spores of unknown origin, in the sea along the Japanese coasts. These spores divide by a wall parallel to the substratum into a lower rhizoidal cell and an upper cell which divides to give a row of cells in which longitudinal divisions appear before long. The records of the formation of similar stages from spores in culture are comparatively few and limited to the very earliest stages. Such records are given by Okamura, Onda, and Higashi (1920) for *P. sub-orbiculata* Kjellm., by Dangeard (1931) for *P. leucosticta* Thuret, and by Kylin (1945) for both *P. umbilicalis* and *P. leucosticta*. Of these investigators Dangeard only is able to state the manner in which spores which germinate in this manner had been formed on the parent-thallus. In this instance they were formed from the entire contents of undivided mother-cells, which are to be found at the edge of the thallus and similar to those described previously by Janczewski (1873) for *P. laciniata* Ag. They may occur on the same thalli as the other type of spore, which is formed by repeated division of the mother-cell or the spermatia. Leafy thalli may originate, therefore, both from spores of unknown origin and from spores liberated from the leafy thalli. It is to be noted that in only one case is the manner of formation of the spores on the parent-thallus known. These spores are either of rare occurrence or else have not been generally recognized; they are said to be asexual in origin.

Berthold (1882), on the other hand, considered that asexual spores were formed as a result of the division of the mother-cell into either two or four spores, both divisions being at right-angles to the surface of the thallus. There appears to be no information as to the manner in which this particular type of asexual spore described by Berthold germinates, nor whether it can be considered as belonging to the same category as those formed singly from the mother-cell. Thus, although rare, two types of asexual spores have been described for the genus *Porphyra*.

A survey of previous investigations shows that the majority of the spores found on thalli of various species of *Porphyra* in all parts of the world are formed, like the spermatia, by repeated division of the mother-cells into a smaller or larger number of spores according to the species. The first wall formed in the mother-cell is parallel to the surface of the thallus. The entire contents of each of the ultimate cells forms a small single spore. On germination such spores give rise to one, two, or three creeping filaments, as has been shown for *P. laciniata* (Janczewski, 1873; Thuret and Bonnet, 1878; Kylin, 1922, 1945), *P. leucosticta* (Janczewski, 1873; Dangeard, 1931; Kylin, 1945), *Porphyra* sp. (Yendo, 1919), *P. tenera* (Okamura, Onda, and Higashi, 1920; Kunieda, 1939), *P. umbilicalis* (Grubb, 1924; Dangeard, 1931). These observations are based entirely on cultures, and identical creeping filaments have not been recorded as occurring in the sea. Filamentous growths found on concrete chippings in the sea by Rees (1940, *a* and *b*) have a slightly greater diameter, but this investigator gives few details and no illustrations, and hence it is impossible to decide how similar they are to those obtained by other investigators in culture. The origin of Rees' filaments is unknown, and he reports that the vegetative cells measure 4–5 μ by 16–20 μ and the liberated spores are from 11 to 14 μ in diameter.

No convincing evidence is available of the formation of reproductive organs on these creeping filaments grown in culture, although on the basis of single examples both Kylin (1922) and Grubb (1924) considered that monospores might be formed. Dangeard (1931), on the other hand, considered that such growths are protonemal in nature, the leafy thalli arising on them as 'pluricellular buds' formed usually from the original spore. Kunieda (1939) considered the filamentous growths pathological, and that such spores liberated from the leafy thallus in the sea pass into a resting state, in which they remain for several months. The evidence provided by Kunieda in support of these statements appears somewhat contradictory, and the weight of evidence provided by other workers is in favour of the contention that germination takes place in the manner stated and without a resting period of more than a few days. The significance of the resulting growths in the life-history is obscure, however, and from this it is clear that a large but vital step in the life-history remains very imperfectly known.

Since the time of Berthold's investigation (1882), spores which germinate in this way have been considered carpospores (= cystocarpospores). Berthold's contention that such is indeed their nature rests on the observation of transverse walls (i.e. walls parallel to the surface of the thallus) in cells still showing what he considers to be fertilization-canals, to which reference will now be made. The justification for considering these spores as carpospores would be the demonstration of fusion of the sexual cells and their nuclei prior to their formation. Observations to be found in the literature relating to sexual fusion fall into two groups. An early investigator, Koschtsug (1872), and a more recent worker, Knox (1926), reported the fusion of spermatia or their derivatives with liberated spores, but these accounts have not been generally accepted. First Berthold (1882) and then Joffé (1896), Dangeard (1927), Kunieda (1939), and Magne (1952) have described what they consider to be the union between spermatia and certain cells of the thallus, not readily distinguishable by morphological features, but called carpogonia. According to Berthold (l.c.) and Dangeard (l.c.) a fine tube grows out from the spermatium and penetrates to the carpogonium, but Kunieda (l.c.) considers that the spermatium is engulfed by the trichogyne-like protuberance of the carpogonium. As this author found fine tubes connecting small bodies on the surface of the thallus with vegetative cells, he considered that what Dangeard took to be fertilization was in fact the spores of an Oomycete, parasitizing the *Porphyra*. This same criticism could be levelled at the accounts of other investigators. Joffé (l.c.), while confirming Berthold's observations, stated that in other cases the egg-cell puts out a filamentous protuberance with which the spermatium fuses. In considering the figures of spermatia given by Dangeard, the difference in size between those figured separately from the thallus and those attached is considerable. The former appear to be genuine spermatia. In this connexion it should be noted that the bodies figured by Ishikawa (1921, Pl. XII, Figs. 10 and 11) on the surface of the thallus are undoubtedly spermatia, as is obvious from their highly characteristic contents, but there are no fertilization-tubes between them and cells of the thallus. Although not of significance one way or the other in proof of the point, it is of interest to notice that the method of fertilization described by Berthold and others is quite different from fertilization in any other algae. Berthold and Joffé also found that these fertilization-canals are much more obvious if the wall of the *living* female cell is swollen with dilute glycerine to two or three times its normal diameter, which fact supports the theory that these tubes are not fertilization-canals but fungal filaments made more obvious by the shrinkage of the cell-contents.

Cytological evidence in support of fertilization is limited to the observation of two nuclei in a cell by Joffé (1896) and to a figure given by Dangeard (1927, Fig. 12*b*) and one by Magne (1952, Fig. *d*). Dangeard's figure shows two adjacent cells to one of which a fertilization-canal is attached. In this cell a smaller and a larger nucleus are in contact and in the neighbouring cell there is a single larger nucleus, interpreted by Dangeard as a fusion-nucleus. Magne (1952) figures four minute bodies which have stained with Feulgen's reagent in a fertilization-canal.

This survey shows that convincing evidence of fertilization is still needed, and it would seem best to avoid the use of the term carpospore for the time being. It has long been known that such spores may form a continuous band around the edge of the thallus (Naegeli, 1847, and Janczewski, 1873), and the writer finds this to be the case very commonly in *P. umbilicalis* var. *laciniata*. If such spores are indeed carpospores, then every cell in the peripheral region of such thalli must be a functional carpogonium, and, moreover, every carpogonium must be fertilized. Doubts as to the sexual origin of these spores is also cast by Janczewski's (1873) observations that different parts of the same mother-cell of *P. leucosticta* may give rise to spores of this type and also spermatia.

Cytological evidence relating to meiosis is not only equally scanty and unconvincing, but also contradictory. Dangeard (1927, Fig. 12*c*) illustrates a stage in the first division of the zygote nucleus of *P. umbilicalis* f. *linearis* considered typical of a meiotic division. On the other hand, Magne (1952, Fig. *e*) considers that there is no reduction at this stage and figures the prophase of the second nuclear division of the 'zygote' in *P. linearis* stained with Feulgen's reagent. The 'chromosomes' are situated in a body, which by its position appears to be the pyrenoid.

This survey makes it clear that only by resorting to purely arbitrary methods of choice between the various statements could a life-history diagram of *Porphyra* be constructed. In addition, no help is to be obtained by comparison with other members of the Rhodophyceae. The evidence relating to critical stages in the life-history is, in most instances, incomplete (being based on a single example in some instances), unconvincing, and contradictory. The following are the only well-substantiated facts regarding the life-history which can be extracted from this unusually large body of literature:

1. The leafy *Porphyra* thallus originates from a unicellular spore, which germinates in a bi-polar manner.
2. There appear to be two categories of spore from which the leafy thallus may develop:
    - (*a*) Spores of unknown origin, which appear to be abundant in the sea at the beginning of the season, when *Porphyra* reappears in the intertidal zone.
    - (*b*) Spores produced by the leafy thalli.
3. Spores of the type of 2 (*b*) are known to be formed in one species, singly from the entire contents of the mother-cells, which resemble the vegetative cells and occur at the edge of the thallus. They are said to be rare and to be formed on the same thalli as the spermatia and the other type of spores (see 5).
4. Certain other spores are formed by the division of mother-cells into two or four spores by walls at right-angles to the surface of the thallus. Their method of germination is not known.
5. Most commonly, either the greater part or the entire length of the thallus liberates spores, which are formed by the repeated division of the mother-cells into eight, thirty-two, sixty-four, or more spores. The first wall in the mother-cells is parallel to the surface of the thallus.
6. These spores germinate laterally to give one, two, or three narrow, creeping, septate filaments.
7. Spermatia are formed by the repeated division of mother-cells situated near the edge of the thallus.

Many of these facts were known before 1880. Other statements in the literature dealing with the life-history of *Porphyra* could be summarized thus:

1. Fertilization is described by some authors as being effected by fusion between an undifferentiated or only slightly differentiated cell of the thallus and a liberated spermatium, and by others as taking place between liberated spores and spermatia.
2. Kunieda, amongst those supporting the former theory, considers that the resulting carpospores pass into a long period of rest, but other writers hold that they germinate at once as in 6 above.
3. The creeping, septate filaments resulting from these spores are regarded by some as an adelophycean phase, possibly giving rise to monospores germinating to form the leafy thallus, but by Dangeard as a protonema on which the leafy thallus arises as a 'bud'.
4. Rees found growths of unknown origin in the sea, but near a prolific growth of *Porphyra*, and somewhat resembling the creeping filaments of 3.

He considered that monospores were formed on them and these gave rise to the leafy thalli.

3. MATERIAL AND METHODS

Most of the thalli used in this investigation have been collected from Rhosneigr on the west coast of Anglesey. The intertidal zone of this shore consists of rocky outcrops and isolated boulders in large stretches of sand. *Porphyra umbilicalis* var. *laciniata* grows on the rocks, boulders, and pebbles, and is a conspicuous and abundant alga in the mid-tide zone at all times of year whereas only rare specimens of *P. umbilicalis* var. *umbilicalis* are found. The boulders, which may be 3 ft. in height and on which the *P. umbilicalis* var. *laciniata* grows, are liable to be completely or partially buried at times by movement of the sand during storms. On similar shores in south Wales *P. umbilicalis* var. *laciniata* has been found either partly or completely buried up to 6 in. below the level of the sand, and it is of interest to note that completely buried thalli are indistinguishable from the fully exposed thalli.

Owing to the uncertainty regarding the specific limits of the *P. umbilicalis* assemblage, care has been taken to use plants of one type only. The attachment is basal, the base of the thallus is cordate or suborbicular, and the thallus itself lacinate to varying degrees. Some thalli reach a length of 50 cm. and a width of 30 cm., but reproductive organs are to be found on quite small thalli. Those bearing spermatia are characterized by yellowish-white edges and those bearing spores by rosy edges, the sterile parts of the thalli being an olive-green. Occasionally both spermatia and spores are formed on the same thallus, the junction of the two areas, being a straight line. The spermatia are formed by repeated division of the mother-cell and the vast majority of the spores develop in the same way. The first wall to be formed is parallel to the surface of the thallus. These spores usually form a completely continuous band around the edge of the thallus, but sometimes thalli are to be found where they occur in small local patches only. Another type of spore formed from the entire contents of the mother-cell has been seen on rare occasions. These develop from the peripheral cells but never in large numbers. Although many hundreds of spores have come under observation, amoeboid movement has been seen in two batches of spores only. Another noticeable feature of both these batches of spores was their great variety of size. Spores usually remain spherical after rounding up in the mucilage and are surrounded at an early stage by a wall or a very strong membrane.

An abundant supply of the desired spores can be obtained by cutting pieces from the edges of suitable thalli and putting them on slides in covered glass dishes in which the atmosphere is kept humid. The pieces of thalli are kept flooded with culture-solution and after 3–5 days many spores are liberated and can be transferred by means of a capillary tube to the experimental shell surface or vessel. The freed spores are exceedingly heavy and if shaken up in a cylinder with sea-water sink very rapidly. Spores liberated from thalli, kept in 2-litre beakers, are deposited at intervals on the bottom of the beaker in sufficient numbers to be visible to the naked eye.

Experiments having the 'infection' of whole shells as their object have been carried out in 2-litre beakers, resting on a wire tray, submerged 3¼ in. below the water-level in a water-bath, 2 ft. by 3 ft. in area. A flow of tap-water through the water-bath has been maintained through the day, but as the temperature of both the tap-water and the room has shown considerable variation, only a very limited control of temperature has been achieved. During the experiments to be described the temperature has fluctuated between 10° and 15° C. apart from the summer period (from the middle of May until towards the end of September), when the temperature of the bath has been between 15° and 20° C. The water-bath has been covered with sheets of vita-glass, and above this five 40-watt fluorescent tubes, 2 ft. long, have been suspended at such a height to give a light-intensity of 3,000 lux at the level of the tray. The cultures have been illuminated by light from these tubes for 12 hours daily, the switch being controlled by an electric clock. Since the apparatus has been against a west window, this artificial lighting has been supplemented by varying and at times appreciable amounts of daylight.

The culture-solution in such beakers has been aerated continuously through porcelain cylinders fed from an electrically operated pulsating diaphragm type of air-pump.

The following culture-solution has been used:

Filtered sea-water from English Channel, 3 miles off shore from Plymouth	1 litre
Soil extract	50 c.c.
Sodium nitrate, $NaNO_3$	0·1 g.
Sodium phosphate, Na_2HPO_4	0·02 g.
0·2% boric acid, H_3BO_3	0·5 c.c.
0·1% manganese sulphate, $MnSO_4 4H_2O$	0·375 c.c.
1% ferric citrate scales	0·625 c.c.

Throughout experiments the culture-solution in the beakers has been replaced at intervals of from 14 to 21 days as proved convenient.

Some experiments have been carried out in Petri dishes, as is described later on (p. 195). In such experiments both the light-intensity and the temperature have been higher than in the beakers as the dishes have been kept on a tray just touching the surface of the water in the water-bath and hence nearer the light. Such cultures have not been aerated, but as the proportion of surface to volume in the culture-solution has been high and the solution has been changed either daily or on alternate days, this has had no apparent deleterious effect.

Autoclaved oyster-shell, either whole or in the form of thin transparent flakes, has been used most commonly as the experimental substratum. Exceedingly thin flakes are easily obtained and the living *Conchocelis* can be observed in them with surprising clarity.

Many of the observations here recorded and many of the photographs reproduced have been obtained from living material in flakes of shell. For more detailed work shells containing the *Conchocelis*-phase have been fixed either complete or in fragments in a mixture of 100 c.c. of 70 per cent. alcohol and 6 c.c. of 40 per cent. formaldehyde. Flakes from such shells have been mounted in the usual way in glycerine jelly, containing gentian violet. Such preparations are excellent for general observations as the stain gradually penetrates the alga, which then contrasts with the white shell matrix in which it is embedded.

Other pieces of shell have been decalcified in Perenyi's[1] fluid as recommended by Bornet and Flahauet (1889). The mat of *Conchocelis* filaments left after such treatment has then been teased apart after transference to glycerine and mounted in glycerine jelly. Alternatively the undisturbed material has been embedded in paraffin and sectioned in the usual way. Nuclear stains have then been employed.

4. EXPERIMENTS TO DEMONSTRATE THE DEVELOPMENT OF *CONCHOCELIS ROSEA* BATTERS FROM SPORES OF *PORPHYRA UMBILICALIS* (L.). KÜTZ. VAR. *LACINIATA* (LIGHTF.) J. AG.

The main conclusion to be drawn from this investigation is that the alga known as *Conchocelis rosea* Batters (1892) is not an autonomous species but a phase in the life-history of *Porphyra umbilicalis* var. *laciniata*. This relationship between two entities showing such profound morphological and physiological differences is very unexpected, but it is also a very essential link in our knowledge of the life-history of the latter. For these reasons the experiments from which this conclusion is drawn are described. It should be noted that the information could not have been obtained in any other way, suggesting that more general application of this method in investigations of life-histories of algae is desirable. While the experiments are set out under numbered headings for clarity, the numbers do not always reflect their chronological sequence.

Experiment 1

In September 1948 spores of *P. umbilicalis* var. *laciniata* from Wembury, south Devon, were germinated on microscope slides submerged in shallow dishes of culture solution. Since the culture tank was not available at that time the dishes were kept out of doors in positions where they received a certain amount of sunshine during the day. The method of germination and subsequent development of these spores agreed completely with the accounts given by previous investigators, Janczewski (1873), Thuret and Bornet (1878), Yendo (1919), Okamura, Onda, and Higashi (1920), Kylin (1922, 1945), Grubb (1924), Dangeard (1927, 1931), and Kunieda (1939), for various species of *Porphyra*. However, for the sake of comparison with what follows, the germination will be described briefly. The centrally placed plastid of the liberated spore moves to a parietal position before the germination of the

[1] 10·0% nitric acid, 4 parts; 90·0% alcohol, 3 parts; 0·5% chromic acid, 3 parts.

spore, which usually takes place within 4 or 5 days. A narrow germ-tube protrudes from one side (Text-fig. 1, *k*, *l*; Pl. X, Figs. 1, 2, 3) and a lobe of the plastid often extends into it (Pl. X, Fig. 2). A second and even a third germ-tube follow fairly quickly and these elongate into sparsely branched tortuous septate filaments, 2·5–6·5 μ in diameter (Text-fig. 1, *d*, *e*, *f*). The first filament usually grows along the surface of the glass slide, but some of those which develop later grow obliquely upwards. Their direction of growth changes frequently, sometimes a circular course being followed and right-angle bends are frequent. Growth is very irregular, and while some branches are long, others are short, and on the surface of some cells protuberances resembling undeveloped branch initials appear. Occasionally larger cells of irregular shape and dense contents are found. It is not unusual for the original spore to divide by a transverse wall (Text-fig. 1, *g*). The plastid in the spore often enlarges and becomes very deeply pigmented, whereas the plastids in the filaments are pale. Thel atter are parietal (Pl. X, Figs. 1, 2, 3; Text-fig. 1, *l*,) and may or may not contain a pyrenoid.

Two features of the germlings in these cultures which appeared to be exceptional and significant were (1) the similarity of young germ-tubes to those of fungal spores, (2) the generally abnormal appearance of the older filaments. These features suggested the need of a specific host or substratum for normal growth, and an experiment with the provision of substrates other than glass was therefore undertaken. It should be remarked, however, that such free-living filamentous growths can be maintained and continue to grow indefinitely, provided the culture-solution is renewed regularly. No spore formation has been seen, however.

Experiment 2

Spores of *P. umbilicalis* var. *laciniata* collected from Rhosneigr, Anglesey, at the end of November 1948, were transferred on November 30 and December 1, either to sterilized pebbles, similar to those on which the adult plants were growing, or to sterilized shells of various kinds. Pebbles and shells were chosen since the adult plant is usually found growing either on rocks or stones or occasionally on barnacles.[1]

As a control, some spores were placed on glass slides as in expt. 1, and these germinated in the manner just described. The pebbles and shells were kept in 2-litre beakers in the culture-tank described on p. 191, but as the tank equipment was being assembled during the course of this experiment conditions varied considerably and the light-intensity during the first weeks was low.

Very few of the spores placed on pebbles put out a germ-tube, and such germ-tubes as did develop were broad, contorted, and deformed and remained very short. On the other hand, the spores enlarged considerably, often doubling their original size and becoming thick-walled. The contents of the

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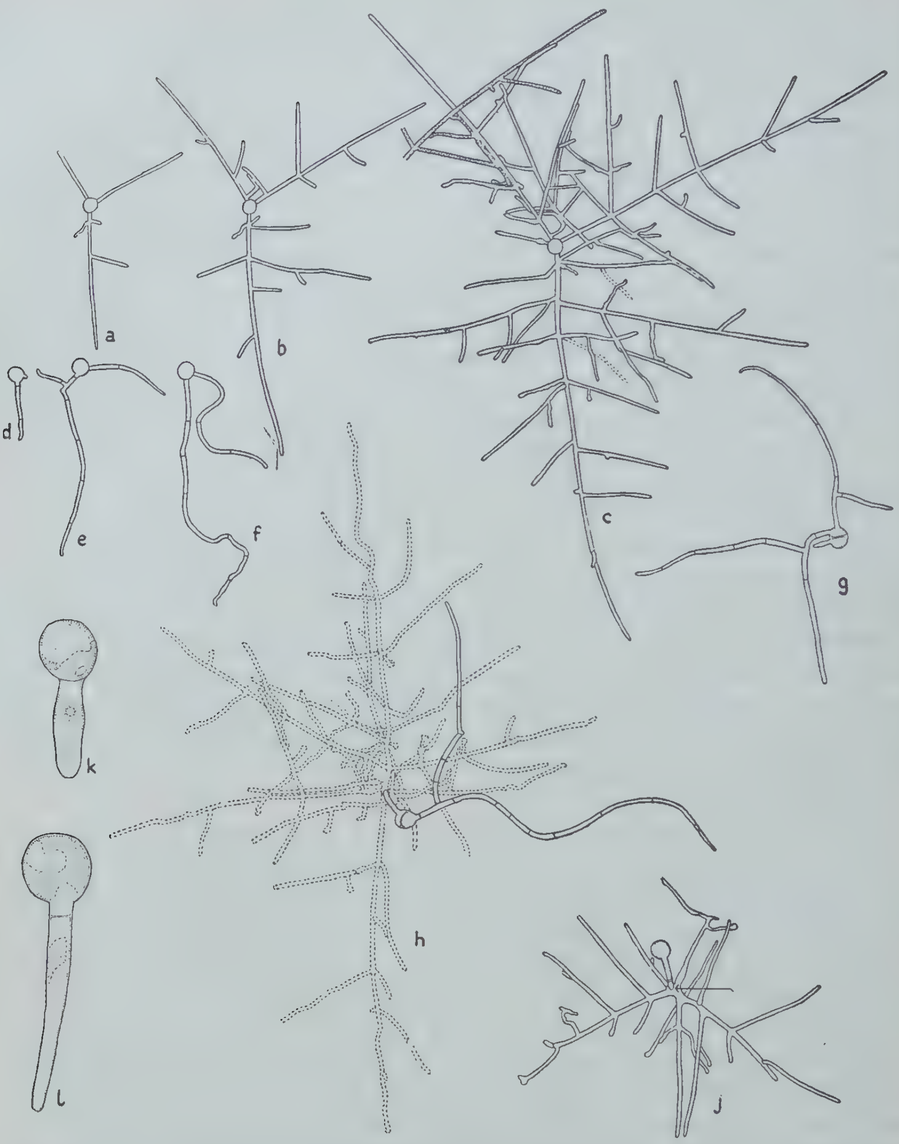
[1] *P. umbilicalis* will also grow on a variety of materials not usually found in the sea, including iron, wood, and concrete. Other species of *Porphyra* are known to be epiphytic.



Text-fig. 1, *a–l*. Development of germlings of the *Conchocelis*-phase. *a, b, c*. Development of germling in shell, 8, 11, and 15 days after liberation of the spore. In *c*, fusions are to be seen both with branches of the germling itself, and with filaments of a neighbouring germling, indicated by dotting. (×105.) *d, e, f*. Germlings growing free from shell. Spores liberated on same day as that of *a, b,* and *c* and growing under otherwise identical conditions. *d*, 8 days, *e*, 11 days, and *f*, 17 days later. (×105.) *g*. Germling on glass, showing division of spore (×125). *h*. Germling which has given rise to two filaments, one of which has penetrated underlying shell (shown beyond point of entry by interrupted line) and the other has remained outside (shown by a continuous line). (×125.) *j*. Germling showing penetration of shell a short distance from spore. Point of penetration indicated by arrow. (×125.) *k, l*. Early stages in development of germlings on glass, showing parietal plastid and pyrenoid. (×400.)

spores increased in density and the colour of the plastids, which divided several times and became parietal, deepened considerably. After some weeks these spores appeared to be still healthy and resembled the resting spores described by Kunieda (1939) for *P. tenera* Kjellm. Since their behaviour is so different from that of spores placed on shells, it seems reasonable to suppose that it is abnormal, however.

The spores placed on sterile shells germinated and on February 28, 1949, i.e. 2 months after the beginning of the experiment, a pink area was noticed in an oyster-shell. Microscopic examination showed that the growth was entirely inside the shell and was without doubt identifiable as *Conchocelis rosea* Batters. The appearance of the highly distinctive fertile cell-rows of *C. rosea* in this shell by June 7 provided further confirmation of the identification. Fertile cell-rows were abundant at the time the experiment was discontinued in July. Soon after the first appearance of *C. rosea* in this culture, its occurrence in the shells of this culture became general.

Certain points of resemblance between *C. rosea* and the germlings of expt. 1 suggested that a connexion between *C. rosea* and *P. umbilicalis* var. *laciniata* could be possible, and so other experiments were planned with the object of settling this point. These fall into two categories: (1) direct observation of the penetration of the shell by germ-tubes from spores of *P. umbilicalis* var. *laciniata*, and (2) the presence or absence of *C. rosea* in shells after contact with either spores of *P. umbilicalis* var. *laciniata* or filaments grown from such spores.

Experiments 3a, 3b, 3c

Experiments which were devised to observe the germination of spores of *P. umbilicalis* var. *laciniata* on shell and the penetration of the shell by the germ-tubes are grouped together here. In the first experiment (3*a*), shells were ground up into pieces smaller than 5 mm. in diameter and set in agar on microscope slides. Spores liberated from *P. umbilicalis* var. *laciniata* collected at Rhosneigr on March 29, 1949, were transferred to these slides, which were kept in a horizontal position in 2-litre beakers in the culture-tank. Many of the pieces of shell were completely pink with *C. rosea* within 2 months, but the actual process of penetration was found impossible to follow as the shell fragments were opaque. Another type of experiment was therefore devised and this proved entirely successful. Perspex or glass rings, 1·5 cm. in diameter, were set in a thin layer of plain 2 per cent. agar in Petri dishes to form small culture chambers. One or sometimes two very thin transparent flakes of oyster-shell were put in each chamber and covered with culture-solution. Spores of *P. umbilicalis* var. *laciniata* were then introduced with the aid of a fine pipette, and the growth and development of certain chosen germinating spores were recorded day by day by means of either drawings or photographs. The first of these experiments (3*b*) was carried out in May and June 1949 and the second (3*c*) in February and March 1950, the spores used originating from thalli of *P. umbilicalis* var. *laciniata* collected at Rhosneigr, in both cases. This proved a very suitable method for observing the germination of the spores, and it was possible to show conclusively that the germ-tubes penetrated the shell flakes and developed rapidly into extensive growths of *C. rosea* (Drew, 1949). Some of the flakes were fixed at various times after 'infection', but others were kept in culture for several weeks. In such cultures both fertile cell-rows and 'plantlets' developed later. Such an experiment is easily carried out and can be repeated at will. The observations made during expts. 3*b* and 3*c* form the natural start of the description of the *Conchocelis*-phase of *P. umbilicalis* var. *laciniata* and so will be deferred until Section 5.

Experiment 4

In order to demonstrate that *C. rosea* develops in sterile shells only after contact with spores of *P. umbilicalis* var. *laciniata*, or as will be shown in expt. 5 after contact with filaments derived from them, spores liberated from thalli collected from Anglesey on March 29, 1949, were put on sterilized shells in 2-litre beakers on April 4. Similar sterilized shells were put in another 2-litre beaker and kept under conditions similar to the former, but no spores were added. By the end of the month growths of *C. rosea* were visible in the shells on which the spores of *P. umbilicalis* var. *laciniata* had been placed, and with the aid of a dissecting microscope it was possible to ascertain that germ-tubes from the spores of *P. umbilicalis* var. *laciniata* were attached to the shell immediately above growths of *C. rosea*. On the following July 16 the first fertile cell-rows were found in these shells, and on December 14, 1949, 'plantlets', to be referred to later, were found in one of the 'infected' oyster-shells. By contrast, no growths of *C. rosea* appeared in the shells of the second beaker, showing that *C. rosea* appears in shells only after contact with spores of *P. umbilicalis* var. *laciniata*. In further confirmation, it should be added that spores of *P. umbilicalis* var. *laciniata* have been put on sterilized shells on several occasions and *C. rosea* has developed in almost every shell so treated. Shells used include limpet, razor, cockle, winkle, and most commonly oyster. The last mentioned shell has proved the most suitable medium as flakes are readily removed from old shells and, being more or less transparent, the contained growths can be easily studied *in situ*, either before or after fixation. This shell-inhabiting phase of *P. umbilicalis* var. *laciniata* has proved very easy to culture and much more luxuriant growths have been obtained than have been found in nature. Under good conditions, growths are visible with a hand lens within 3 weeks of the 'infection' of the shells and without magnification within 4 weeks.

Experiment 5

Further confirmation of the connexion between *C. rosea* and *P. umbilicalis* var. *laciniata* was obtained by bringing flakes of sterilized oyster-shell and fragments of hen's egg-shell into contact with free-living filamentous growths, obtained from spores of *P. umbilicalis* var. *laciniata* liberated on April 1 and 2, 1949. The spores used were from the same plants as those used in expt. 4.

Two months after germination these filamentous growths were well developed and the first sterile shell-flakes were placed on them. Filaments penetrated the flakes quickly and according to the size of the flakes (all were less than 1 cm. long and only a few mm. broad) filled them with *C. rosea* within 6 to 8 weeks. In one flake of marine shell, fertile cell-rows developed within 2 months. In the case of three other marine shell-flakes superficial 'plantlets' were present 2½ months after penetration by the first filaments.

Growth in hen's egg-shell was good, as is shown by Figs. 9 and 10 of Pl. IX. The fragments of shell in Fig. 9 were photographed 7½ weeks after they had been placed in contact with free-living filaments derived from spores of *P. umbilicalis* var. *laciniata* and that of Fig. 10 after 13½ weeks. Growth of *C. rosea* is normal in this type of shell, but as the shell is opaque it is not a suitable medium in which to grow *C. rosea* for detailed observation. In addition, tufts of such filamentous growths were put inside sterilized hen's egg-shells submerged in culture solution in 2-litre beakers on July 18, and by September 8 filaments had grown to the outside of the shell in sufficient number to give a growth visible to the naked eye.

The results of these experiments prove conclusively that *C. rosea* originates from spores of *P. umbilicalis* var. *laciniata* and represents a phase in the life-history of that alga.

5. THE *CONCHOCELIS*-PHASE UNDER CONDITIONS OF CULTURE

Although *C. rosea* occurs occasionally in the intertidal belt, it is usually found by dredging in water up to 32 metres in depth. It is known from many European shores as well as from the Atlantic and Pacific seaboards of the United States. *C. rosea* grows in a variety of shells as well as in the calcareous tubes of *Spirobis* and *Pomatocerous* and the calcareous plates of a stalked barnacle. It has also been found in *Lithothamnion laevigata* and calcareous stone. Although a widespread and commonly occurring organism, *Conchocelis* has not been studied frequently. Apart from references to its distribution the only accounts are those of Batters (1892) and Rosenvinge (1931). Its unusual features have made its classification difficult, but Batters (l.c.), with the prior approval of Bornet, classed it in the Porphyraceae. Rosenvinge (l.c.), however, transferred it to the Nemalionales on account of the presence of what he considered pit-connexions of the Floridean type.

The following account is based entirely on material grown in culture.

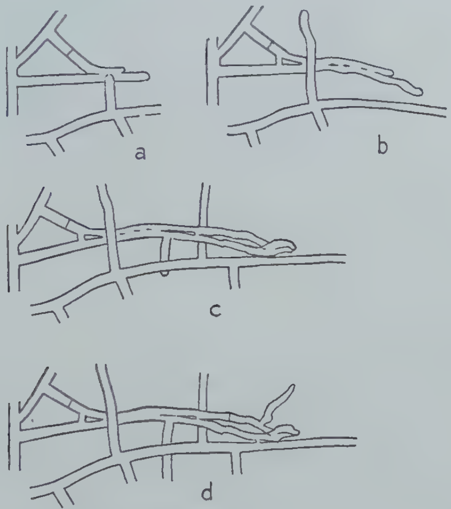
5 (*a*). *Vegetative development*

When spores of *P. umbilicalis* var. *laciniata* are placed on flakes of shell, as described in expts. 3*b* and 3*c*, there is a high percentage of germination (Pl. X, Figs. 4 and 5) and each spore puts out one or two germ-tubes. In many instances, however, the germ-tube arises on the under side of the spore where it touches the shell and as it penetrates the shell at that point the process cannot be seen (Text-fig. 1, *a*, *b*, *c*, and Pl. X, Figs. 6, 7, 8). Some spores give rise to two germ-tubes which grow over the surface of the shell to varying lengths before penetrating it (Pl. X, Fig. 9), while some are found to be still free from the shell after a considerable interval of time (Text-fig. 1, *h*). In other instances only lateral branches of the original filament from the spore have been seen to penetrate the shell, and in such cases, one spore may 'infect' the shell in several places and give rise to growths over a considerable area. The actual penetration of the shell is easily demonstrated when it takes place at a short distance from the spore, as is shown by the examples of Pl. XI, Figs. 1 and 2, and Text-fig. 1, *j*.

Once inside the shell, the filament grows in a straight line, just beneath and parallel to the surface of the shell. Branches arise on either side of the main filament and also grow parallel to the surface. In addition, other main filaments develop from the point of entry. Young growths therefore have a characteristic, more or less pinnately branched appearance (Text-fig. 1, *a*, *b*, *c*, and Pl. X, Figs. 6, 7, 8), which contrasts with the irregular form and branching of all but the youngest filaments growing away from or before entering the shells (Text-fig. 1, *d*, *e*, *f*). This is shown very clearly in Text-fig. 1, *h*, where filaments in the shell are indicated by an interrupted line and those outside by a continuous line. Whereas the external portions of germlings which have penetrated shell and filaments of germlings growing on glass are septate, filaments inside shell are non-septate until a comparatively late stage, cf. Text-fig. 1, *h*. Directly a filament penetrates the shell, a marked change in the appearance of the cell-contents takes place immediately, for whereas the plastid in the cells of the external portion of the filaments is parietal and may contain a pyrenoid, beyond the point of entry, the cell-contents appear homogeneously pink and it is not only impossible to detect discrete plastids but pyrenoids are absent. The straight filaments of the germlings as well as those in older growths have diameters ranging from 2·0 to 5·0 μ, but the majority have a diameter of 3·0 μ.

Under the conditions in the culture-apparatus, growth of germlings in the shell has been good. One such germling photographed 8, 11, and 14 days after liberation of the spore is shown in Figs. 6, 7, and 8 of Pl. X respectively. The germling of Pl. X, Fig. 10, photographed in the living condition is of the same age as that of Fig. 8. The rate of growth of germlings inside the shell is much more rapid than that of germlings away from shell but otherwise under dentical conditions. This is illustrated by Text-fig. 1, *a*–*f*. Text-fig. 1, *a*, *b*, and *c* are of a germling in shell, 8, 11, and 15 days after liberation of the spore and *d*, *e*, and *f* of germlings away from shell, 8, 11, and 17 days after liberation. The shells of Pl. IX, Figs. 1–3, are shown at intervals of 13 and 16 days, respectively, Fig. 1 being a photograph of the shells 2 months after spores were brought into contact with them. Pl. IX, Figs. 4, 5, 6, and 7, show a limpet shell inside which spores of *P. umbilicalis* var. *laciniata* were put on May 25, 1949. Pl. IX, Fig. 4, shows the extent of the *Conchocelis*-growths on August 17, and Fig. 5 on September 26, Figs. 6 and 7 show the development of *Conchocelis* on the other side of the shell on November 4 and December 9.

Although in the early stages the system of branching filaments develops mainly in a plane parallel to and near the surface of the shell, a few branches soon penetrate deeper into the shell material. Some of the shell flakes kept under observation were penetrated in the first instance on the lower side, and the development of such branches could be watched growing through the shell. Just before reaching the upper surface, they were observed to turn at right-angles and grow parallel to the surface of the shell. Once this position had been reached, pinnate branch systems, such as is characteristic of very young germlings, developed from these branches.



TEXT-FIG. 2, *a–d*. Fusing filaments of the *Conchocelis*-phase drawn June 9, 10, 13, and 16, 1949. (×225.)

A very unexpected characteristic of the filaments of *Conchocelis* growing in shell is the frequency with which they fuse with each other. In the case of germlings, it is clear that fusions take place between filaments of not only the same, but also of separate germlings (Text-fig. 1, *c*). These fusions may take place where two filaments meet more or less at right-angles, in which case the growth of one or other filament may be arrested (Pl. XI, Fig. 5 to left). Where two filaments are developing side by side fusion results by the gradual enlargement of numerous small connexions formed between the two (Pl. XI, Figs. 3, 5). A scissor type of fusion is formed when two filaments grow obliquely towards each other and after complete and fairly wide fusion each apex continues on its way (Pl. XI, Fig. 4). The capacity to fuse appears to be lost a short way behind the growing apex; thus if the tip of a young filament touches the older part of another filament, the former continues to grow either along or up and over the other filament and then straight ahead. Septa occasionally develop in the neighbourhood of fusions (Pl. XI, Fig. 3). Text-fig. 2, *a–d*, shows the growth of a pair of fusing filaments at various intervals of time. Such fusions are not common in the algae, but *Colaconema* is another genus for which anastomoses are known (Batters, 1896). Chemin (1926) has shown that, like

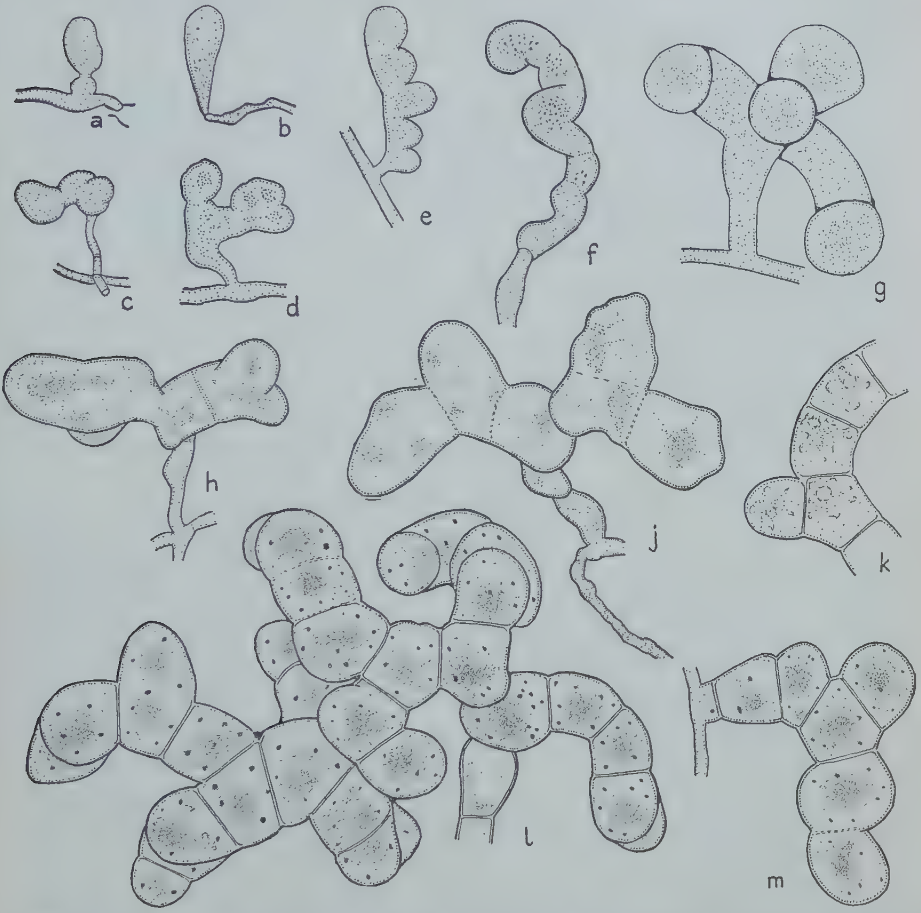
Conchocelis, spores of *Colaconema Bonnemaisoniae* are able to germinate away from their usual substrate, and further investigation may show that this alga also is but one phase in a life-history.

As branches become more numerous in any given area of shell, massed fusions as in Pl. XI, Fig. 6, are to be seen, and farther from the apex of the filaments more and more short convoluted branches, of irregular shape and varying diameter, appear. Fusions between these branches are frequent and at first a network results, but as the process continues the appearance is more like that of a jig-saw puzzle with the constituent pieces pulled very slightly apart. The longer filaments in such dense growths are somewhat tortuous and of varying diameter, and contrast with the straight filaments which are so conspicuous in the early stages of colonization of an area. Since straight filaments are absent in densely filled areas, it seems possible that they become tortuous and of varied diameter as they age. Eventually, and particularly near the surfaces of the shell, a dense growth of filaments results (Pl. XI, Figs. 7 and 8). After decalcification of such material by Perenyi's fluid (p. 192) a mat of filaments remains.

5 (*b*). *Fertile cell-rows*

In every experiment (six groups in all) in which the *Conchocelis*-phase of *P. umbilicalis* var. *laciniata* has been cultured in shells or flakes of shell, structures similar to those described by Batters (1892) as inflations and Rosenvinge (1931) as fertile cell-rows have developed in at least some of the shells or flakes. Under most favourable conditions, these fertile cell-rows begin to appear 2 to 3 months after the 'infection' of the shells. Due to the time needed to search a shell completely, the frequency of their occurrence has not been estimated but in some samples taken at random; fertile cell-rows have been exceedingly abundant, while in others they have been entirely absent. When observed *in situ* in the shell matrix, most mature fertile cell-rows are seen to consist of rosettes of branched filaments. Some of these rosettes are much more compact and the constituent filaments more branched than others. Their size is also variable, but 150 μ is an average diameter. The filaments comprising the rosette ascend obliquely across the layers of the shell and thus differ from the narrow vegetative filaments, the majority of which grow parallel to the layers of the shell (Pl. XII, Fig. 1).

The development of the fertile cell-rows has been followed not only in material still embedded in the shell but also in material decalcified after fixation and then teased apart before mounting in glycerine jelly. The fertile cell-rows originate as irregularly shaped, contorted swellings on the vegetative filaments, usually as lateral branches but occasionally as the terminal portions of filaments (Text-fig. 3, *a–d*). These swellings reach a considerable size and may branch before any transverse walls develop (Text-fig. 3, *f–h*, and Pl. XII, Fig. 2). At first the contents of these swollen cells are dense and homogeneous (Text-fig. 3, *a–f*), but with increasing age their colour deepens and becomes localized in definite plastids (Text-fig. 3, *h*, *j*) which develop at intervals along the central axes of the initials, which are usually branched at that stage. Ultimately transverse walls develop, but they are never as thick as the peripheral walls. Sometimes there are indications of the presence of a special area in the centre of each transverse wall, but the details of its structure remain obscure. The cells of the mature cell-rows are rounded and usually slightly longer than broad. Their diameter is 17·0–18·0 μ, but can vary from 13·0 to 21·0 μ.



TEXT-FIG. 3, *a–m*. Development of fertile cell-rows of the *Conchocelis*-phase. *a–e*. Young initials showing connexion with vegetative filaments and homogeneous cell-contents. *f*, *g*. Later stages, *g* showing well-developed branches. *h*, *j*. Formation of transverse walls and plastids. *k*. Portion of mature fertile cell-row showing cells with central plastid with pyrenoid. *l*, *m*. Mature fertile cell-rows. (×600.)

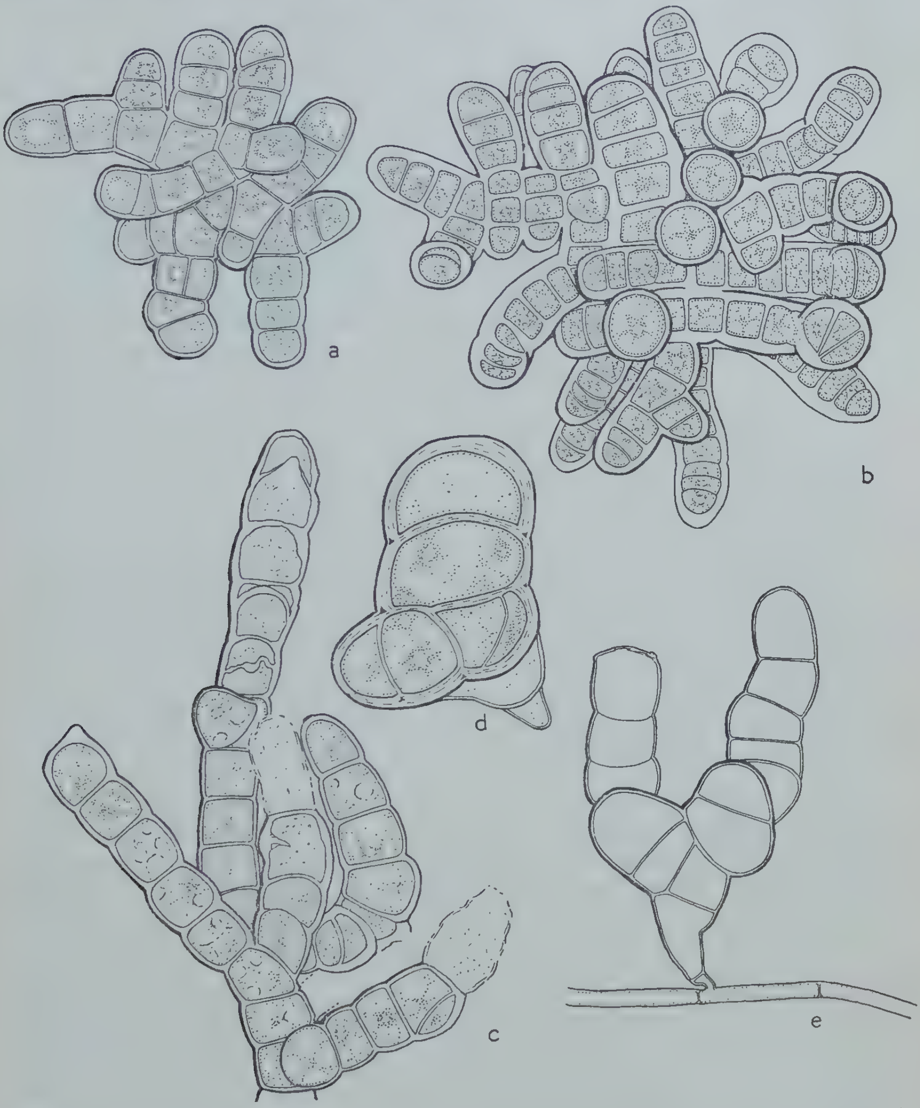
In the material grown in culture, no indication has been seen of the rounding up of the contents of the cells of the fertile cell-rows to form spores, but this has been described in naturally occurring material (Batters, 1892; Rosenvinge, 1931; Jao, 1937; Drew and Richards, 1953). Rosenvinge (l.c.) and

Jao (l.c.) figure such spores escaping from the cells laterally, although it is difficult to see how this can happen in a shell matrix. Drew and Richards (l.c.), on the other hand, found evidence of the breakdown of the transverse walls after the formation of the spores (a feature figured by Rosenvinge also) and their subsequent movement along the resulting tube to the surface of the shell where they are presumably liberated.

5 (*c*). *'Plantlets'*

In addition to the fertile cell-rows, structures now to be described under the general term of 'plantlets' have also occurred in the cultures. They have developed during four separate experiments, in two of which they appeared internally in interstices in whole shells (expt. 4, p. 196, and a similar experiment carried out 2 months later). In the other two experiments (expt. 3, p. 195, and expt. 5, p. 196) the 'plantlets' developed on or at the surface of small flakes of shell. Some of the material thus obtained was used for investigating the origin of the 'plantlets' and their structure, while the remainder was cultured in an effort to induce further development of the 'plantlets' and the possible production of spores. These experiments were unsuccessful, the 'plantlets' becoming more and more moribund without any conclusive evidence of spore formation.

The 'plantlets' occurring in the interstices of whole shells were first noticed with the naked eye when they floated into the culture solution when a piece of oyster-shell containing a dense growth of *Conchocelis* was being prized open for examination of fertile cell-rows. More than a dozen 'plantlets' escaped at one fracture, and 5 months later another part of the same shell was found to contain numerous similar 'plantlets'. Occasionally these 'plantlets' remain *in situ* (Pl. XII, Fig. 3) when a shell is broken, and it is probable that only a small percentage of the 'plantlets' which actually developed in such shells have been found. 'Plantlets' which develop in the interstices of whole shells are more or less flat disc-like structures with a diameter which may reach 350·0 μ. They consist of filamentous projections from a small central pseudo-parenchymatous area. Occasionally, as in the case of the 'plantlet' of Pl. XII, Fig. 3, this is well developed. The general appearance of the 'plantlet' depends considerably on the number and length as well as the degree of branching of the filamentous projections, all of which characters are very variable (cf. Pl. XII, Figs. 3, 6, and 8, and Text-fig. 4, *a*, *b*). Both longitudinal and oblique divisions take place in these filaments, but there is no indication whether growth is apical or intercalary. Sometimes the apical cells appear moribund, but others divide to give groups of cells (Text-fig. 4, *b*). The features of the individual cells are very constant, however. The external walls are comparatively thick, although the transverse walls are thin. Each cell contains a centrally placed plastid with a pyrenoid, so that the cell structure is similar to that of *P. umbilicalis* var. *laciniata* and to the fertile cell-rows of the *Conchocelis*-phase (Pl. XII, Fig. 3). The cells have a diameter ranging from 20·0 to 30·0 μ, but the majority are 25·0 μ in diameter. The length of the cells of the filamentous portions are usually only half their diameter, although in the centre of the disc where cell divisions may have ceased the cells are quadrate. Measurements may therefore vary from 8·0 to 22·0 μ. Clear refringent bodies occur in some of these cells, but their nature is unknown (Pl. XII, Fig. 8).



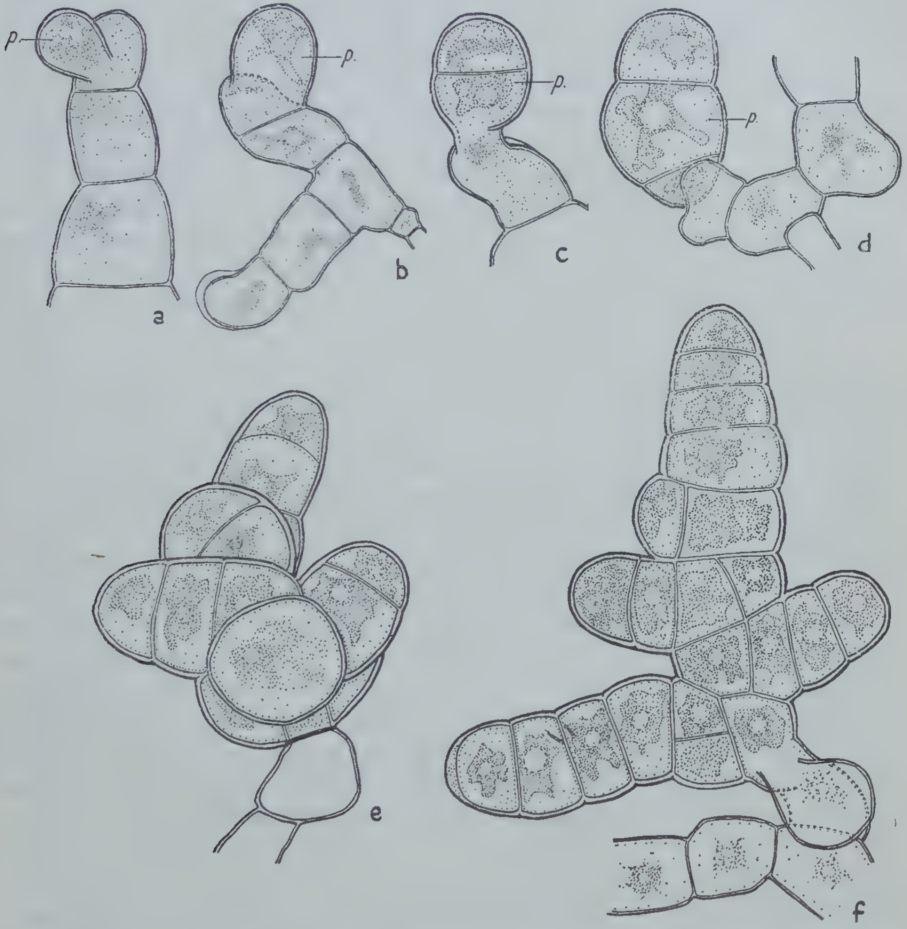
TEXT-FIG. 4, *a–e*. *a*, *b*. 'Plantlets' from interstices in shell. *c*. Part of filament of 'plantlet' developed at surface of flake of shell. *d*, *e*. Growths on filaments on agar. (All × 300.)

The 'plantlets' which developed on the surface of the shell flakes of expt. 5 were comparatively few, and when examined in the living condition had the appearance of clusters of short, thick filaments, growing obliquely to the surface of the shell (Pl. XII, Fig. 7). On the other hand, those of expt. 3*c* occurred in five separate culture chambers and, in addition, were numerous and well developed. Many of these 'plantlets' were of considerable size, for example, that of Pl. XII, Fig. 5, would occupy the whole page if magnified to the same extent as that of Pl. XII, Fig. 3. The under surface of one shell flake was crowded with 'plantlets' of all sizes. These 'plantlets' appeared to consist of tufts of short sparingly branched filaments when viewed alive *in situ* (Pl. XII, Figs. 4 and 5) and to be essentially the same as those of expt. 5. Some resembled the more filamentous 'plantlets' found in the interstices of whole shells, and on breaking up one of these flakes it was found that parenchymatous 'plantlets' had developed between the layers of the flake and that filamentous projections had grown beyond the surface of the shell and appeared as superficial growths. Several of these growths were unattached and floated away into the culture solution as the flake was separated into its constituent layers (Pl. XII, Fig. 4). Examination of fixed material has shown that the cell structure of these 'plantlets' is the same as that of the 'plantlets' which developed in the interstices of whole shells (cf. Pl. XII, Figs. 3 and 9), but many of the transverse walls show structure resembling pit-connexions (Pl. XII, Fig. 9). The diameter of the cells of these 'plantlets' has varied from 22·0 to 32·0 μ, but it is usually near 27·0 μ and the length is about two-thirds of the diameter. It has not been possible to determine whether the bases of the very tufted and entirely superficial, filamentous 'plantlets' are pseudoparenchymatous or not as it is impossible to do so without applying pressure, which then separates the tufts too extensively.

No spore formation has been seen on the 'plantlets', empty cells in their filaments having the appearance of dead cells rather than cells which have liberated their contents.

The structures described as 'plantlets' do not all appear to have originated in the same way. Some of those which have developed on the surface of the flakes of shell have been found to be in connexion with fertile cell-rows inside the shell. A flake of shell from expt. 5 was decalcified after fixation, and the resulting mass teased apart and mounted in glycerine jelly. As a result, all stages in the development of 'plantlets' from fertile cell-rows were found and some of these are illustrated in Text-fig. 5, *a–f*. The mass of material from another decalcified flake from this experiment was embedded and sectioned, and in Fig. 12 of Pl. XII three protuberances from fertile cell-rows appear in the section. On the other hand, 'plantlets' have occurred on flakes in which fertile cell-rows were absent and another 'plantlet' growing on the edge of a flake of shell was clearly attached to a narrow cell at its base. The origin of 'plantlets' in interstices of whole shells is unknown.

The writer has not found similar 'plantlets' in material of *C. rosea* from the sea and it is not clear whether records in the literature refer to the same structures. Batters (1892), for example, on p. 27, states that inflations (i.e. fertile cell-rows) often 'became detached from the horizontal filaments, and are then capable of an independent existence' and also that he found them in shells without any sign of the horizontal filaments. Rosenvinge (1931, p. 621) also found complexes of about fifty fertile cells, but was unable to detect continuity with the narrow vegetative filaments. The example figured by Rosenvinge (Fig. 618 G) is much smaller and more filamentous than those which developed in the present experimental work and the individual cells are smaller.



Text-fig. 5, *a–f*. Stages in development of 'plantlets' at surface of flake of shell from cells of fertile cell-rows. *p* = 'plantlet'-initial. (All ×600.)

A record of quite another kind is given by Rees (1940, *a* and *b*), who describes the occurrence of 'plantlets' in an account of the life-history of *P. umbilicalis*, but as no figures are given, a closer comparison with the 'plantlets' of these cultures is not possible. The 'plantlets' described by Rees (1940, *b*) were free-living entities growing on concrete chippings attached to concrete walls in the sea. In another paper (1940, *a*) these microscopic 'plantlets' are said to be 4–14 cells long and to have two or three short branches. The cells measure 16·0–20·0 by 4·0–5·0 μ and spores liberated from them are 11·0–14·0 μ in diameter. These he assumes give rise to the leafy thalli of *P. umbilicalis*, but essential links showing the connexion between the two are not given.

Dangeard (1931) suggested that the *Porphyra* thallus arises as a bud on the filamentous growths he obtained from spores of the same type as those which develop into the *Conchocelis*-phase. The present writer has also seen small growths on free-living *Conchocelis*-filaments, but all of these have had the appearance of abnormalities, such as the one figured in Text-fig. 4, *d*. It is interesting to note one of the largest and also most normal in appearance (Text-fig. 4, *e*) showed some resemblance to a fertile cell-row.

While it appears that the 'plantlets' described as occurring in these cultures are probably normal growths, yet without further evidence regarding their origin and their fate, their significance in relation to the life-history of *Porphyra umbilicalis* var. *laciniata* cannot be assessed at present.

6. DISCUSSION

The experimental work, recorded in the preceding pages, has dealt with the germination of certain spores of *P. umbilicalis* var. *laciniata* and the growths developed from them. The spores are of the type referred to under heading 6 (p. 189) in the summary of established facts concerning the life-history of species of *Porphyra*. This work has shown that such spores give rise to filaments which, while capable of growing for long periods in liquid culture-solution, show considerably more rapid and extensive growth in a shell matrix. In such a matrix they develop into extensive colonies, identifiable with the 'species' *Conchocelis rosea* Batters, which is known to have a wide distribution in the infra-littoral regions of the sea. Fertile cell-rows, the only reproductive organs reported previously for *C. rosea*, have occurred in the cultures and in addition 'plantlets' have developed both in interstices in shells and on surfaces of flakes of shell. In certain instances these 'plantlets' have arisen by the continued growth of fertile cell-rows, but in other cases there has been no such connexion.

This information indicates that the life-history of species of the genus *Porphyra* is much more complex than previously supposed. Much remains unknown, however, and at this stage in the investigation it is not possible either to assess the significance of the *Conchocelis*-phase or to interpret the relationship between the leafy thallus and the shell-inhabiting filamentous form, in terms of gametophyte and sporophyte, for example. Such considerations must await the results of investigations of the cytology of both the *Porphyra* thallus and the *Conchocelis*-phase, of the method of origin of the spores from which the *Conchocelis*-phase develops, and also of the fate of the contents of the fertile cell-rows and of the 'plantlets'. The establishment of the connexion between *P. umbilicalis* var. *laciniata* and *C. rosea* has shown that remarkable and profound morphological and physiological differences can exist between phases in the life-history of a single species.

In the case of the genus *Porphyra* the physiological differences appear to be of ecological significance in the life-history. It has long been known that some species are only found in the intertidal zone during certain months of the year; for example, in the British Isles, *P. linearis* Grev. occurs between November and May only. The form in which such species exist and the places where they are to be found during the remainder of the year have been matters of speculation, but it would appear likely that they exist as the *Conchocelis*-phase in deep water while conditions are unfavourable to growth in the intertidal zone. In culture at any rate, the *Conchocelis*-phase can be considered perennial. The mechanism by which an intertidal phase is re-established from a phase living in deep water is a problem still to be investigated, but it is possible that storms and off-shore currents play a part, as *Conchocelis*-bearing shells have been found in the intertidal zone, sometimes wedged between rocks on which *P. umbilicalis* var. *laciniata* has been growing. In addition, an examination of the sand of the Rhosneigr shore showed the presence of filaments of *Conchocelis* in minute pieces of shell. As some species of *Porphyra* occur regularly on barnacles, the feeding habits of the latter may also be of significance.

Several very distinctive features of the *Conchocelis*-phase are exceptional and worthy of note. (1) The capacity of the filaments to grow in either liquid culture-solution or in shell shows unusual powers of adaption and may increase the chances of survival of germlings until contact with shell is established. (2) It is clear that filaments inside shell show physiological adaption to that environment, as marked changes take place in the cell-contents directly the shell is penetrated. In fact, whereas the external portions are septate and the cells contain a discrete plastid sometimes with a pyrenoid, the internal portions are non-septate for a considerable time and the plastids are either ill defined or else the pigments are diffused throughout the protoplasm. (3) The fusions which take place between *Conchocelis*-filaments of the same and different plants are of quite another type to those known in the *Rhodophyta* (except possibly for *Colaconema*), but show much greater resemblance to fusions which occur between fungal hyphae. (4) The structure of the differentiated areas, which are sometimes visible in the transverse walls and most easily seen in the fertile cell-rows and 'plantlets', are in need of further investigation before their identity with pit-connexions of the Florideae can be accepted. In some instances the resemblance to pit-connexions seems to be due to the transverse walls remaining incomplete. (5) The method of development and the structure of the mature fertile cell-rows are quite unlike those of any other reproductive structures described for the algae, and until their significance is elucidated by further investigation, it seems preferable to continue the use of this non-committal name, fertile cell-rows, given to them by Rosenvinge (1931).

Results obtained under conditions of culture are open to the criticism that they may not represent what happens under natural conditions. There is the additional complication, in this instance, that in culture spores from thalli of various species of *Porphyra* can give rise to filaments able to survive for some time away from contact with shell. It is necessary therefore to assess the degree of reliance which can be placed on the experiments recorded. Although such small filaments might be overlooked, the fact that such free-living filaments have not been found in the sea, whereas the shell-inhabiting form has been reported frequently as *Conchocelis rosea*, provides convincing support in favour of the shell-inhabiting form being the natural one and the free-living form being the result of culture conditions. This conclusion is further supported by their more rapid growth in shell and the abundant development of fertile cell-rows. In contrast, the records of reproductive organs on the free-living filaments is limited to single records by Kylin (1921) and Grubb (1924), neither of which are satisfying. Dangeard (1931) considered that *Porphyra* thalli might develop from 'buds' which he observed on the free-living filaments. From the present writer's observations these would appear to be more in the nature of abnormalities or abortive attempts to form fertile cell-rows, such as have been recorded in this paper also. In the light of the facts here established, Kunieda's (1939) assertion that spores of *P. tenera* similar to the ones used in this work pass into a resting stage is probably incorrect and the result of the conditions under which they were cultured. He considered those which germinated to give creeping filaments to be pathological, but it has been shown to what degree this is true.

Preliminary experiments on the germination of spores from thalli of *P. linearis* Grev. and from an unidentified species of *Porphyra* which grows epiphytically on *Fucus* during the early summer months in Anglesey, have shown that they also give rise to a *Conchocelis*-phase.

7. SUMMARY

1. A survey of the literature is given showing how incomplete is our knowledge of the life-history of species of *Porphyra*.

2. As the first part of a renewed investigation, and using *P. umbilicalis* (L.) Kütz. var. *laciniata* (Lightf.) J. Ag. as material, one type of spore has been germinated in culture.

3. On glass and in liquid culture-solution, such spores give rise to creeping septate filaments, capable of surviving for some time but showing no reproductive cells.

4. Experiments are described which show that when such spores or the filaments which develop from them are brought into contact with shell, either the filaments or the germ-tubes from the spores penetrate the shell and give rise to growths identical with the 'species' *Conchocelis rosea* Batters. The latter is known from the infra-littoral regions of the sea in many parts of the world.

5. *C. rosea* is therefore a phase in the life-history of *P. umbilicalis* var. *laciniata* and not an autonomous species. Outstanding features of the *Conchocelis*-phase are described, including fusions between filaments.

6. Fertile cell-rows similar to those described in naturally occurring material have developed in the shells in culture, but no formation of spores has been seen.

7. 'Plantlets' are also described, the cells of which show great similarity to the cells of *Porphyra*. They have developed in interstices in shells and on the surface of flakes of shell.

8. The interpretation of the relationship of these very different morphological and physiological phases in the life-history of *P. umbilicalis* var. *laciniata* will have to wait the completion of further stages in the investigation.

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATES

Illustrating K. M. Drew's article on 'Studies in the Bangioideae. III. The Life-history of *Porphyra umbilicalis* (L.) Kütz. var. *laciniata* (Lightf.) J. Ag. A. The *Conchocelis*-phase in culture.'

PLATE IX

The Conchocelis-phase in shells

1. Shells 2 months after spores of *P. umbilicalis* var. *laciniata* placed on them, showing numerous points of 'infection'.

2. Same 13 days after 1.

3. Same 29 days after 1.

4. Limpet shell 12 weeks after spores of *P. umbilicalis* var. *laciniata* placed on it, showing area occupied by *Conchocelis*-phase.

5. Same 6 weeks after 4. Small dark spots, colonies of diatom.

6. Reverse side of same shell, 11 weeks after 4.

7. As 6, but 16 weeks after 4.

8. Three-month-old growth of *Conchocelis*-phase in oyster-shell.

9. Fragments of hen's egg-shell, 7½ weeks after contact with free-living filaments of *Conchocelis*.

10. Similar fragment 13½ weeks after contact.

11. Growth of *Conchocelis* in shell, 2 months' old.

All nat. size.

PLATE X

Germination of spores of P. umbilicalis var. laciniata

1, 2, 3. Living germlings on glass slides, showing parietal plastid and pyrenoid in side view in middle of Fig. 1. (×400.)

4, 5. Living germlings in shell. (4, ×55; 5, ×165.)

6, 7, 8. Germling in shell, 8, 11, and 14 days respectively, after liberation of spore which is still visible. (×110.)

9. Germling showing division of spore and two points of entry (marked by arrows) into shell.

10. Part of living germling 14 days after liberation of spore. Spore to be seen in centre of photograph. Filaments in various thickness of shell. (×220.)

PLATE XI

Vegetative filaments of the Conchocelis-phase

1, 2. Penetration of shell short distance from spore. Place of entry indicated by arrow (1, ×500; 2, ×440.)

3, 4, 5. Fusions of various types between filaments in shell. (×660.)

6. Fusion of several filaments. (×330.)

7, 8. Portions of shell where growth is becoming dense. (×300.)

PLATE XII

Fertile cell-rows and 'plantlets' of the Conchocelis-phase

1. Mature rosettes of fertile cell-rows in shell. (×150.)

2. Young fertile cell-row before formation of transverse walls. Connexion with vegetative filament visible. (×250.)

3. 'Plantlet' *in situ* after opening of shell. Note central plastid and pyrenoid. (×250.)

4, 5. 'Plantlets' formed on surface of shell flakes. (4, ×27·5; 5, ×55·0.)

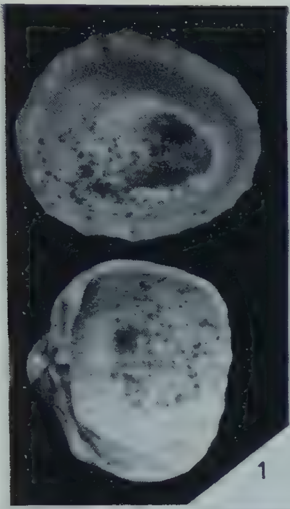
6, 8. 'Plantlets' formed inside shells. (×250.)

7. Early stage in development of 'plantlet' on surface of shell, showing central plastid and pyrenoid in cells. (×250.)

9, 11. Portions of filaments of 'plantlets' such as those of 4 and 5, showing cell-contents. (×250.)

10. Portions of small fertile cell-row showing central plastid and indications of pyrenoid in some cells. (×250.)

12. T.S. of growth of *Conchocelis* after decalcification, showing protuberances from fertile cell-rows beyond surface of shell. (×150.)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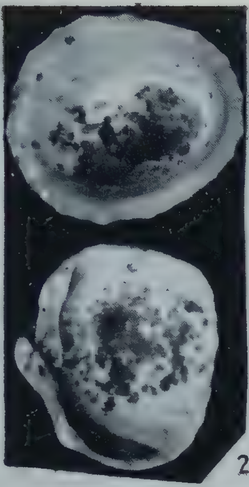
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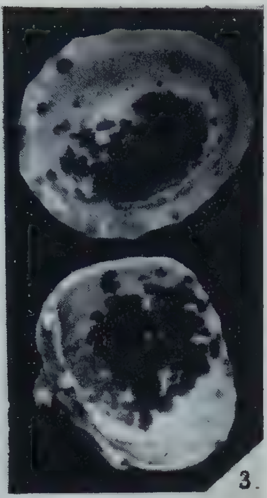
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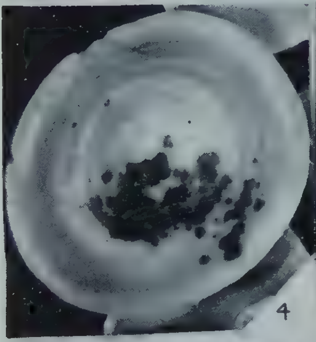
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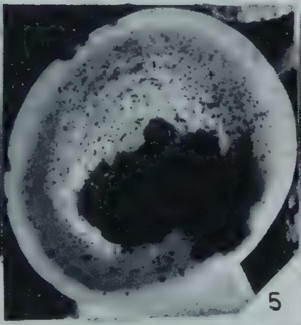
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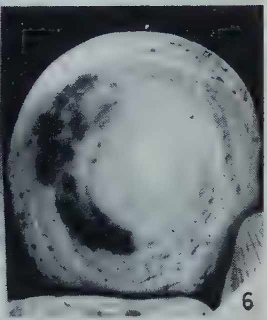
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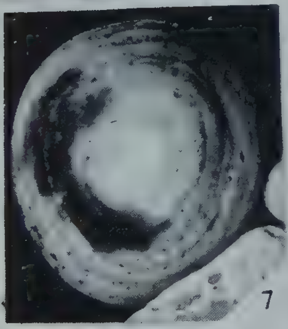
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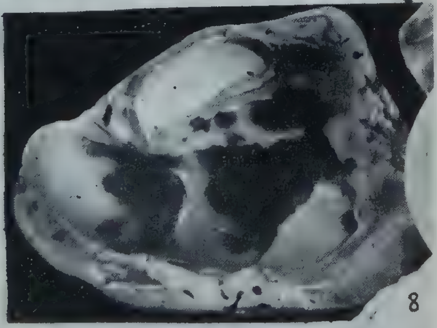
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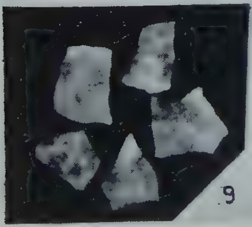
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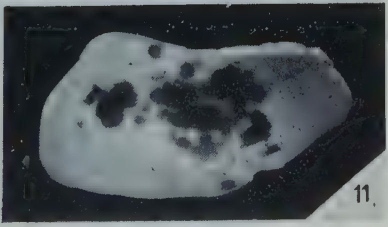
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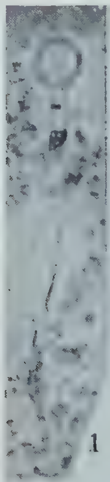
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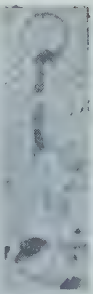
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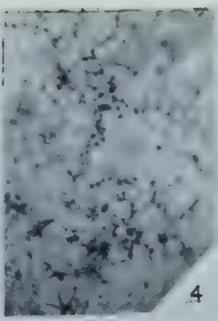
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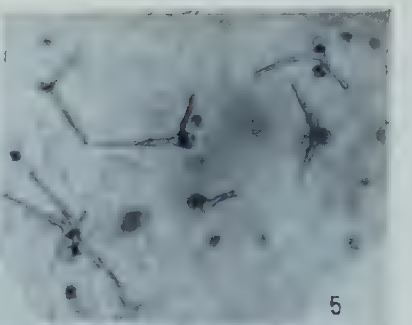
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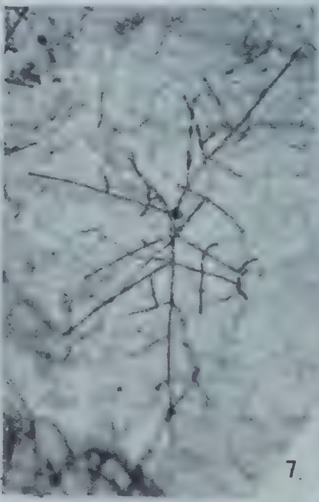
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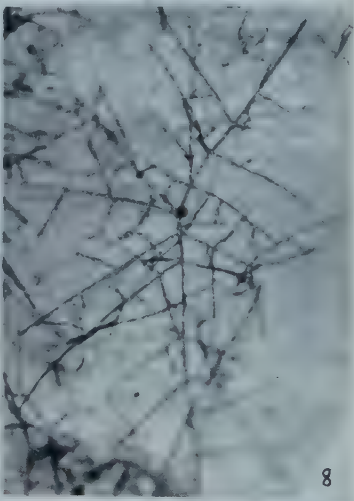
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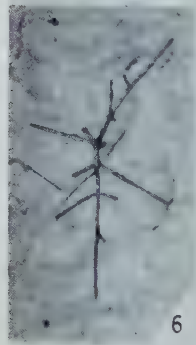
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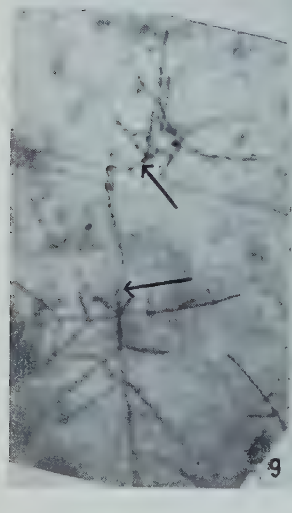
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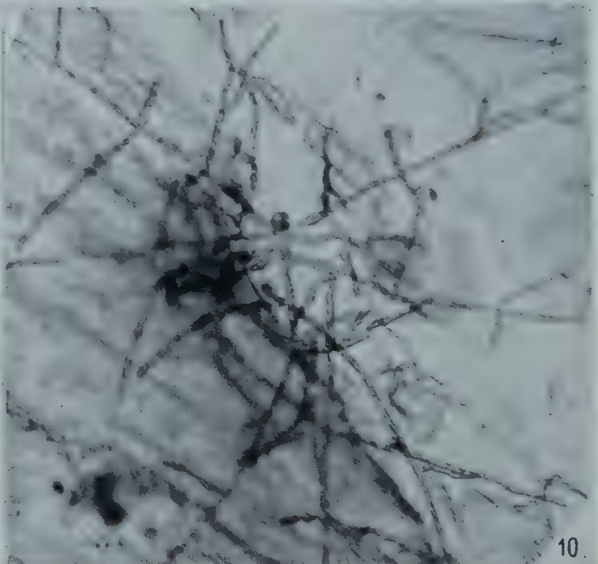
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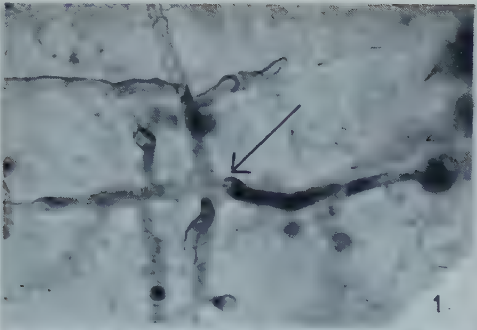
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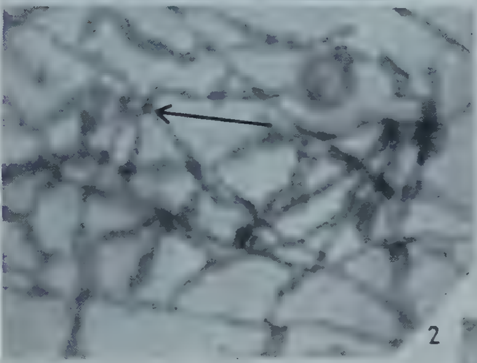
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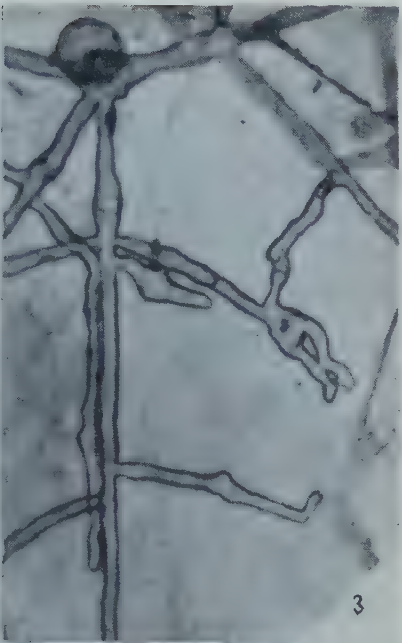
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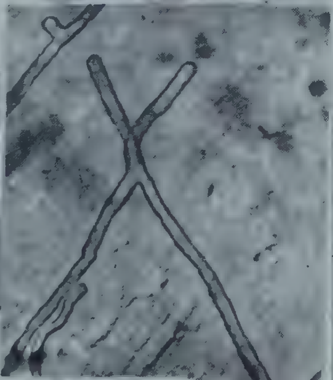
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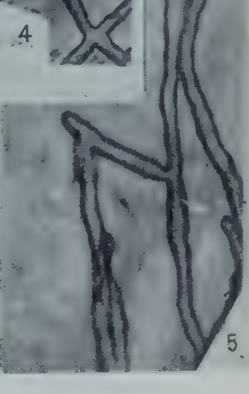
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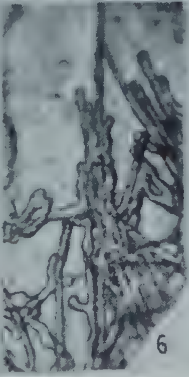
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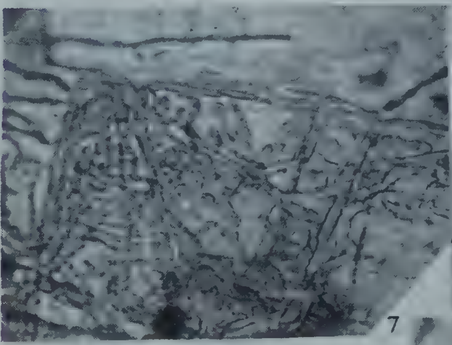
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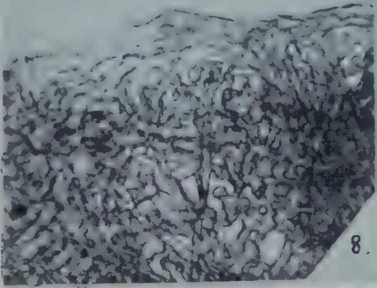
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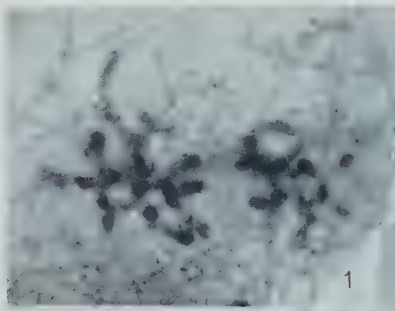
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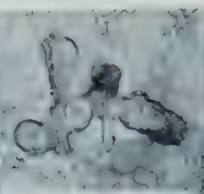
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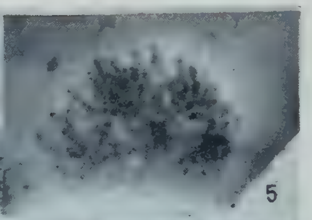
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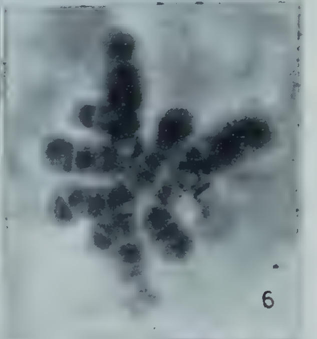
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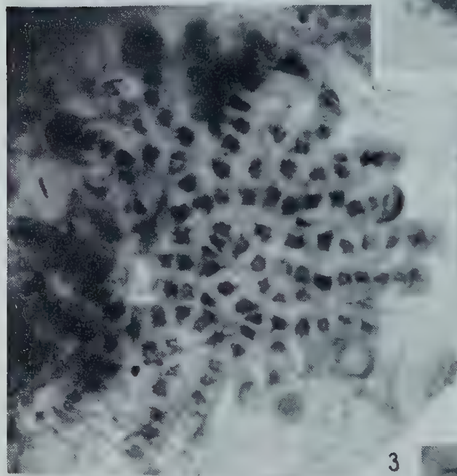
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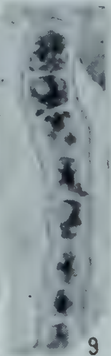
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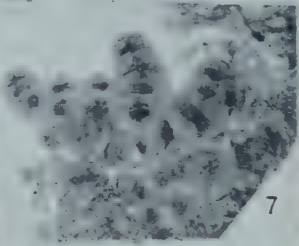
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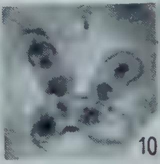
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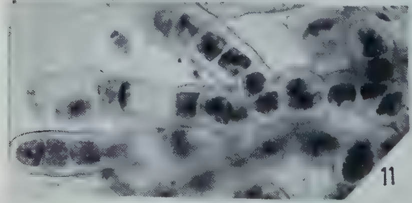
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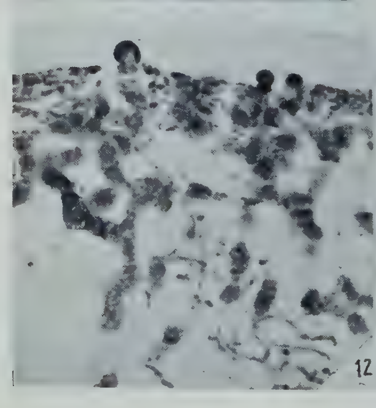
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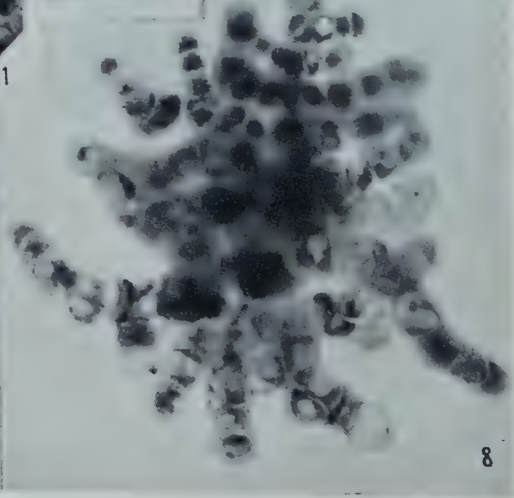
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K. M. DREW

A New Method for determining the Type of Distribution of Plant Individuals

BY

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WITH AN APPENDIX BY

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With one Figure in the Text

ABSTRACT

The method depends on linear measurements between random points and adjacent individuals, and between adjacent pairs of individuals. Its results compare favourably with those of the current methods when tested on synthetic and natural populations. The method is quicker than the quadrat methods and is especially useful for analysing the distribution of trees.

1. INTRODUCTION

A CONSIDERABLE amount of work has been published on the types of distribution of plant individuals. Current methods for determining the type of distribution require the use of quadrats. The quadrat, though extremely valuable to the plant ecologist, is not always very practical to use. The method described in this paper does not require the use of quadrats.

2. CONCEPTS AND TERMS

2.1. *Population* is a term with several distinct statistical and biological meanings. A *population* will be defined as the individuals of a species living together on an area. Any discrete unit (shoot, tiller, frond, &c.) may be used as a *plant individual.*

The *density* of a species is 'the number of individuals per unit area' (Goodall, 1952).

Sample areas are essential for the quantitative analysis of vegetation. The *quadrat* will be defined as a sample area of given shape and size used for analysis within a plant community. Objection may be made to this definition on the grounds that the term is derived from *quadratum* (a square) and was first defined (Clements, 1905) as 'a sq. m. of vegetation marked off for counting, mapping, etc.'. Numerous authors have used the term loosely to include

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all sizes (Clements states that the area need not be 1 sq. m.) and shapes, so that it would seem justifiable to define it as above.

2.2. *Distribution* is static arrangement in space; *dispersion* is a dynamic concept implying dissemination. *Dispersed*, being the past tense, may be applied in some cases (e.g. in the terms *overdispersed* and *underdispersed*) to the results of dispersion.

Three types of distribution have been distinguished, *random*, *aggregate*, and *regular*. A *random*, or 'normal', distribution is one in which the individuals of a population are distributed according to chance. There has been considerable confusion in the terminology of non-random distributions. This has been mainly due to the use of the term *overdispersed* in two opposing meanings. The statistical term of Svedberg (1922) refers to the distribution of quadrats containing 0, 1, 2, 3, . . . individuals; his *overdispersed* population has more empty quadrats than would be expected by chance, and vice versa for his *underdispersed* population. Also there is a physical meaning referring to the actual distribution of the individuals on the area; an overdispersed population has its individuals evenly distributed, and an underdispersed population has its individuals in groups. Because of this confusion, the terms overdispersed and underdispersed will not be used in this paper.

The term overdispersed used in its statistical sense (grouping of individuals) will be replaced by the term *aggregated* as suggested by Goodall (1952), and the term underdispersed used in its statistical sense (evenly distributed individuals) will be replaced by the term *regular*.

3. CURRENT METHODS

3.1. All the current methods involve the counting of the number of individuals present in quadrats which are either thrown at random or arranged in a regular manner.

3.2. If the individuals are randomly distributed, then the proportion of random quadrats containing 0, 1, 2, 3, . . . individuals will be given by the values of the terms of a Poisson series

$$e^{-m}, \quad me^{-m}, \quad m^2e^{-m}/2!, \quad m^3e^{-m}/3!, \ldots,$$

where m is the mean number of individuals per quadrat. Observed and calculated values of these terms are compared, and the significance of the deviations is measured by a χ^2 test.

3.3. Clapham (1936) pointed out that for a Poisson series the variance (V) equalled the mean (M), and hence the *relative variance* (V/M) was unity. For aggregated populations the relative variance is greater than unity, and for regular populations it is less than unity. The significance of the deviation of V/M from unity is measured by a 't' test or a χ^2 test. When a 't' test is used, the value of the standard error (S) is given by $S = \sqrt{[2/(n-1)]}$, where n is the number of observations (Greig-Smith, 1952). The value of χ^2 in the χ^2 test is obtained by multiplying V/M by the number of degrees of freedom $(n-1)$ (Skellam, 1952). When the number of degrees of freedom is large (>30), the normal deviate $[\sqrt{(2\chi^2)}-\sqrt{(2n-1)}]$ is used (Fisher, 1950).

3.4. Greig-Smith (1952) has shown that the values of χ^2 and V/M obtained in the above methods depend on the size of the quadrat employed. Hence in order to determine whether or not a population is distributed at random it may be necessary to use at least two different sizes of quadrat.

4. DESCRIPTION OF THE NEW METHOD

4.1. If I is the distance from an individual chosen at random to the nearest individual, and P is the distance from a point chosen at random to *its* nearest individual, it can be shown (see appendix) that if the individuals are distributed independently and at random the mean values of the squares of I and P are equal. The coefficient of aggregation (A) will be defined as the mean value of the squares of P divided by the mean value of the squares of I.

Hence, for the whole population, when the individuals are distributed at random, A equals unity. If they are aggregated, then A is greater than unity, whilst if they are regularly distributed A is less than unity. The value of A is a measure of the amount of aggregation.

To test the significance of the deviation of an observed value of A from unity, the 'Incomplete Beta-Function' tables (Pearson, 1934) are used; or, where a large number of observations have been taken, the 'Normal Curve Probability Integral' tables (Pearson, 1914).

4.2. The method of computation is as follows:

i. Calculate $\sum(P^2)$ from n observations.
ii. Calculate $\sum(I^2)$ from n observations.
iii. Calculate A from the equation $A=\sum(P^2)/\sum(I^2)$.
iv. Calculate x from the equation $x=A/(1+A)$.

For small numbers of observations ($n \leqslant 50$).

v. Find the value of Ix from the table of the incomplete beta-function (Pearson, 1934).

vi. Ix (or when $A>1$, $1-Ix$) is the probability of a chance departure from expectation ($\frac{1}{2}$) exceeding that observed and in the same direction.

(To ease this computation an abac (Fig. 1) has been constructed. For n observations, the probability (p) of exceeding the calculated value of x by chance can be read off quickly.)

For large numbers of observations ($n>50$).

v. Calculate X from the equation $X=|x-0{\cdot}5|.2\sqrt{(2n+1)}$, where $|x-0{\cdot}5|$ is the absolute value of $x-0{\cdot}5$.

vi. Find the value of $0{\cdot}5(1+a)$ from Pearson's (1914) tables of the normal curve probability integral.

vii. Calculate the probability (p) of exceeding the observed value of A by chance from the equation $p=1-0{\cdot}5\,(1+a)$.

APPLICATION OF THE NEW METHOD TO SYNTHETIC POPULATIONS

5.1. *Methods.* Plant individuals were represented by map pins on a square metre of millimetre graph paper. The position of any point on this area could be given (to the nearest millimetre) by a six-figure grid reference; hence by using six random figures an individual or point could be placed at random.

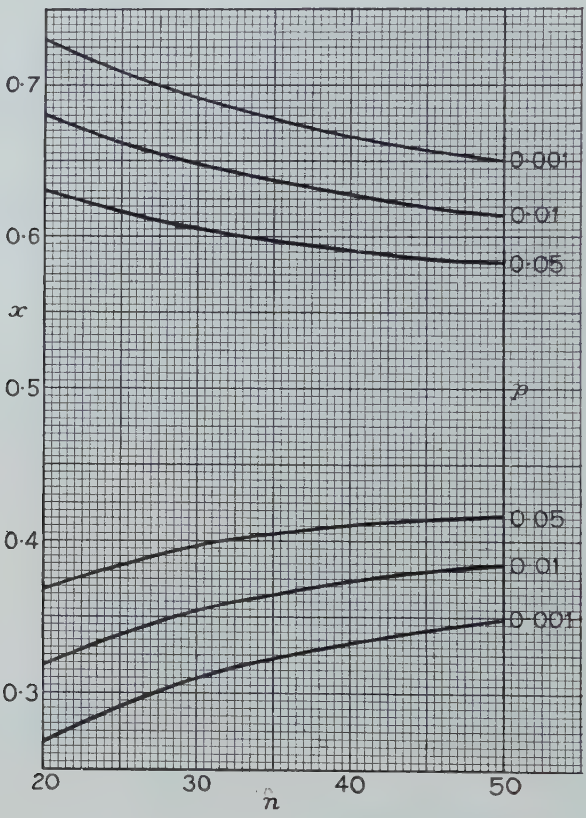

FIG. 1. Abac for determining the probability (p) of exceeding the calculated value of x for n observations by chance.

Nine different populations were used. The details of these are given in Table I.

In the aggregated populations the individuals were placed in hexagons of side 25 mm. containing seven individuals, the central one being placed at random. In the regular distributions the individuals were placed at random except where the position of an individual fell within 25 mm. of one which had already been placed. When this happened, a new random grid reference was selected, and the process repeated until the required number of individuals has been placed.

TABLE I

Details of Synthetic Populations

Population.	Type of distribution.	Total density.	Density per grid unit.
A 1	Random	100	0·25
A 2	,,	200	0·50
A 3	,,	400	1·00
B 1	Aggregated	100	0·25
B 2	,,	200	0·50
B 3	,,	400	1·00
C 1	Regular	100	0·25
C 2	,,	200	0·50
C 3	,,	400	1·00

As the individuals were numbered, random figures were used to obtain random individuals. The distance from a random individual, or point, to its nearest individual was measured by a millimetre scale. In each population 100 measurements of *P* and *I* were made. Later it was decided to leave out the values where the distance measured was greater than the distance from the random individual, or point, to the edge of the area. Hence, in order to keep the number of observations of *P* and *I* the same, some other values had to be discarded.

In order to compare this method with the current methods, the 1 sq. m. area was divided into a grid of 400, 25 sq. cm. quadrats, and the number of individuals present in each was recorded. Contiguous quadrats were grouped into 100 quadrats of 100 sq. cm., 25 quadrats of 400 sq. cm., 16 quadrats of 625 sq. cm., 4 quadrats of 2,500 sq. cm., and the total 10,000 sq. cm. The number of individuals present in these quadrats was obtained from the 25 sq. cm. quadrat data. The relative variance test was done on the values obtained for quadrats of 25, 100, 400, 625, and 2,500 sq. cm. (1, 4, 16, 25, and 100 grid units) and the values for the 25 and 100 sq. cm. quadrats (1 and 4 grid units) were fitted to a Poisson series and tested by a χ^2 test. (It was not possible to fit a Poisson series to quadrats of 400 sq. cm. and over as there were not enough observations. In fitting data to a Poisson series, all the quadrats which have less than five individuals are grouped together.)

5.2. *Results.* The results of the analysis of these synthetic populations are shown in Table II. They do not require explanation except for population C 1. That this regularly distributed population does not differ significantly from a random distribution is due to the method of placing regular individuals and the low density. Had the value of the minimum distance between two adjacent individuals been larger, then the distribution of this population would have been significantly different from random.

6. APPLICATION OF THE NEW METHOD TO NATURAL POPULATIONS

6.1. *Methods.* The method used for natural populations was similar to that used for the synthetic populations. A uniform area of a plant population

TABLE II

Analysis of Synthetic Populations

Quadrat size (grid units).	Relative variance.						Fit to Poisson.			Coefficient of aggregation.		
	V/M	*t*	*p*	χ^2	Normal deviate.	*p*	χ^2	d.f.	*p*	*A*	*n*	*p*
Population A 1												
1	1·0528	0·75	0·4–0·5	420	0·752	0·05–0·06	0·8580	1	0·3–0·5	0·8655	80	0·181
4	0·9899	0·07	> 0·9	98	−0·036	0·96–0·97	1·2608	2	0·5–0·7			
16	0·8125	0·65	0·5–0·6	19·5		0·2–0·3						
25	0·8640	0·37	0·7–0·8	13·0		0·3–0·5						
100	1·0667	0·82	0·4–0·5	3·2		0·3–0·5						
Population A 2												
1	1·1630	2·30	0·02–0·05	464	2·232	0·02–0·03	1·5089	2	0·3–0·5	1·1842	86	0·133
4	1·0505	0·36	0·7–0·8	104	0·386	0·70–0·71	5·5972	4	0·2–0·3			
16	0·9688	0·11	> 0·9	23		0·3–0·5						
25	1·4507	1·23	0·2–0·3	21·8		0·1–0·2						
100	2·2534	1·54	0·2–0·3	6·8		0·05–0·10						
Population A 3												
1	1·0576	0·81	0·4–0·5	422	0·821	0·41–0·42	0·8990	3	0·8–0·9	1·0401	94	0·394
4	1·0404	0·28	0·7–0·8	103	0·317	0·75–0·76	5·8609	7	0·5–0·7			
16	1·0417	0·14	0·8–0·9	25		0·3–0·5						
25	0·9280	0·20	0·8–0·9	13·9		0·3–0·5						
100	0·5600	0·54	0·6–0·7	1·7		0·3–0·5						
Population B 1												
1	2·6968	23·97	< 0·001	1,076	18·149	⩽ 0·001	83·9788	1	< 0·001	16·1499	59	⩽ 0·001
14	5·7980	33·76	< 0·001	574	19·846	⩽ 0·001	90·9395	2	< 0·001			
16	10·2708	32·11	< 0·001	246		< 0·001						
25	6·7733	15·81	< 0·001	101		< 0·001						
100	2·7467	2·14	0·1–0·2	8·2		0·02–0·05						

Population B 2											
1	2·8472	26·09	< 0·001	1,136	19·435	⩽ 0·001	228·6226	2	< 0·001	7·1302	73	⩽ 0·001
4	5·5152	31·77	< 0·001	546	19·010	⩽ 0·001	235·5285	4	< 0·001			
16	9·2292	28·50	< 0·001	222		< 0·001						
25	5·6534	12·75	< 0·001	85		< 0·001						
100	2·9467	2·38	0·1–0·2	8·8		0·02–0·05						
Population B 3												
1	2·6416	23·19	< 0·001	1,054	17·682	⩽ 0·001	169·8342	3	< 0·001	4·9640	89	⩽ 0·001
4	4·4849	24·52	< 0·001	444	15·763	⩽ 0·001	652·8012	7	< 0·001			
16	5·2552	14·74	< 0·001	126		< 0·001						
25	3·5253	6·92	< 0·001	53		< 0·001						
100	3·8867	3·54	0·02–0·05	12		0·001–0·01						
Population C 1												
1	0·8320	2·37	0·01–0·02	332	−2·463	0·01–0·02	6·8861	1	0·001–0·01	0·9342	72	0·341
4	0·7474	1·78	0·05–0·10	74	−1·870	0·06–0·07	2·9347	2	0·2–0·3			
16	0·8125	0·65	0·5–0·6	19·5		0·2–0·3						
25	0·9067	0·26	0·7–0·8	13·6		0·3–0·5						
100	0·4800	0·64	0·5–0·6	1·4		0·3–0·5						
Population C 2												
1	0·7518	3·51	< 0·001	300	−3·736	< 0·001	14·6422	2	< 0·001	0·7835	94	0·026
4	0·6263	2·63	0·001–0·01	62	−2·900	0·001–0·01	10·1179	4	0·001–0·01			
16	0·5417	1·59	0·1–0·2	13		0·02–0·05						
25	0·9600	0·11	> 0·9	14·4		0·5–0·7						
100	0·4934	0·62	0·5–0·6	1·5		0·3–0·5						
Population C 3												
1	0·5815	5·92	< 0·001	232	−6·690	⩽ 0·001	23·1811	3	< 0·001	0·5563	89	< 0·001
4	0·4344	3·98	< 0·001	43	−4·762	< 0·001	33·2879	7	< 0·001			
16	0·3229	2·35	0·02–0·05	7·7		< 0·001						
25	0·7093	0·80	0·4–0·5	10·6		0·2–0·3						
100	0·1333	1·06	0·3–0·4	0·4		0·05–0·10						

was chosen and a 20×20 square grid set up, the length of the side of the grid unit (L.G.U.) depending on the density of the individuals. The selected area was the *site* within which all sampling was done. Every fifth individual encountered, whilst traversing the rows to record the number of individuals in each grid unit, was used as an individual from which the distance to its nearest neighbour was measured.

The setting up of a grid is not necessary in order to determine the type of distribution of a population. A series of lines are placed across the site and every *j*th individual occurring within a short but fixed distance of one or both sides of the line is used as an individual chosen at random.

Random points were chosen by throwing a cane at random. All distances were measured at ground level in the herbaceous populations and at breast height in the tree populations. No allowance was made for the size of the individuals; the distance between them was measured to the nearest centimetre.

6.2. *Populations studied.* Twelve natural populations were analysed. Most of these were from areas known to have been little altered by man, and thought to have remained unchanged for hundreds of years. Hence the distribution of the individuals was assumed to have attained equilibrium with the environment and to be entirely natural. They were selected to obtain a wide range of type of distribution, habitat, and vegetation. However, as the new method will potentially have its greatest use in wooded areas, more than half the examples were chosen from tree populations.

A brief comment on each population will now be given.

P. I. MAYO BOG (*Eriophorum angustifolium*) (July 1951). The site was 4 miles south of Louisburgh, near Westport. It was part of a vast expanse of blanket bog which had presumably been growing throughout the subatlantic period and had apparently been little influenced by man. There was no dominant species; *Narthecium ossifragum*, *Eriophorum*, *angustifolium*, *Rhynchospora alba*, *Schoenus nigricans*, *Molinia caerulea*, and several species of *Sphagnum* were very frequent. There were occasional pools and hummocks on the site, which was similar to, but wetter than, those described by Pearsall and Lind (1941). Fruiting heads of *Eriophorum* were chosen as individuals.

P. II. MAYO BOG (*Utricularia intermedia*) (July 1951). The site was identical with that of P. I; the two analyses were done simultaneously. Flowering heads of *Utricularia* were chosen as individuals. The *Utricularia* grows in bog pools, but only four of the pools on the site contained it, hence it occurred in four clumps. This population was chosen as being an example of apparent extreme aggregation.

P. III. RANNOCH BOG (*Eriophorum angustifolium*) (August 1951). The site was quite close to Rannoch Station, Perthshire, on an area of bog at an altitude of 950 ft., which had probably been burnt a few years before it was sampled. There was no dominant species, and the most frequent species were *Calluna vulgaris*, *Erica tetralix*, *Narthecium ossifragum*, *Trichophorum caespitosum*, *Eriophorum angustifolium*, several species of *Sphagnum*, several Hepaticae, *Cladonia impexa*, and *C. uncialis*. Fruiting heads of *Eriophorum* were chosen as individuals.

P. IV. HILLOCK WOOD (*Fagus sylvatica*) (March 1952). The site was part of a plateau beechwood about 2 miles east of Princes Risborough in the Chiltern Hills. The area had presumably been beechwood since prehistoric times and had been managed (felling and clearing) since the sixteenth century. There were occasional oak trees present and the ground flora was mainly composed of brambles. It would be type 'B' in Watt's (1934) classification. Beech trees were chosen as individuals.

P. V. RIDLEY WOOD (*Fagus sylvatica*) (April 1952). The site was part of a beechwood, with some oak and holly, about 3 miles east of Ringwood in the New Forest. Local records show that the area had been beechwood for at least 350 years. Beech trees were chosen as individuals. (This was the only population of trees in which all the stages from seedlings to trees were present. Beeches with a girth at breast height of 20 cm. or more were regarded as trees.)

P. VI. ROTHIEMURCHUS WOOD (*Pinus sylvestris* ssp. *scotica*) (July 1952). The site was about 4 miles south-east of Aviemore, Inverness-shire. It was a remnant of the old Caledonian pine forest and showed no signs of having been felled for several hundred years. The ground flora consisted mainly of *Vaccinium vitis-idaea*, *V. myrtillus*, *Calluna vulgaris*, and *Hylocomium splendens*. The vegetation is more fully described by Tansley (1949). Pine trees were chosen as individuals.

P. VII. CRESSBROOKDALE WOOD (*Fraxinus excelsior*) (August 1952). The site was about 2 miles east of Miller's Dale, Derbyshire. It was part of a regenerating ashwood on a very steep, unstable, limestone scree slope facing west. Occasional rowan trees were present, and where there was a ground flora it was mainly *Deschampsia caespitosa* or *Mercurialis perennis*. The vegetation of these ashwoods is described by Moss (1913). Ash shoots were chosen as individuals.

P. VIII. WATENDLATH WOOD (*Betula pubescens*) (September 1952). The site was about 5 miles south of Keswick in the Lake District. It was part of a birchwood on a slope facing north at an altitude of 1,000 ft. Pearsall (1950) suggests that originally the area was covered by oakwood which changed to birchwood several hundred years ago. Since then there has been considerable degeneration of the woodland to moorland. Watendlath Wood is a small relic of this once extensive birchwood. Regeneration is now prevented by sheep grazing, and signs of degeneration to bog can be seen in places. The ground flora mainly consisted of grasses and bracken. Birch trees were chosen as individuals.

P. IX. WATENDLATH GRASSLAND (*Pteridium aquilinum*) (September 1952). The site was next to that of P. VIII. It had probably had a similar history but had reached a further stage of degeneration; the wood had been completely replaced by bracken. The bracken was at the 'mature' stage in Watt's (1947) terminology. Bracken fronds were chosen as individuals.

P. X. South Bentley Wood (*Quercus petraea*) (October 1952). The site was part of an enclosed oakwood, with a considerable amount of beech, about 5 miles north-west of Lyndhurst in the New Forest. Local records show that it had been oakwood for several hundred years. The site was chosen in an area of considerable regeneration, and oak saplings (there were no trees on the site) were chosen as individuals.

P. XI. Cothill Fen (*Schoenus nigricans*) (October 1952). The site was in a *Schoenus* dominated area of Cothill Fen, about 5 miles south-west of Oxford. The site was calcareously flushed and all the *Schoenus* occurred as large tussocks, which were chosen as individuals. It was the area where Dawkins (1939) analysed the distribution of *Schoenus*. As the size of the tussocks was not negligible compared with the distance between them, the distances were measured from the centre of tussocks and at tussock height.

P. XII. Kingley Vale Wood (*Taxus baccata*) (November 1952). The site was part of a yewwood in Kingley Vale National Nature Reserve, about 5 miles north-west of Chichester in the South Downs. The wood was at the head of a chalk coomb on a steep slope facing south. The area has been described by Watt (1926), who showed that it had been yewwood for several hundred years. Yew trees were chosen as individuals.

6.3. *Results*. The results of the analysis of these natural populations are shown in Tables III and IV.

TABLE III

Details of Natural Populations

No.	Locality.	Species.	L.G.U. (m.)	Area of site (sq. m.)	Total density.	Density per grid unit.
I	Mayo Bog	*Eriophorum angustifolium*	1·0	400	510	1·275
II	Mayo Bog	*Utricularia intermedia*	1·0	400	101	0·253
III	Rannoch Bog	*Eriophorum angustifolium*	1·0	400	91	0·228
IV	Hillock Wood	*Fagus sylvatica*	5·0	10,000	382	0·955
V	Ridley Wood	*Fagus sylvatica*	10·0	40,000	192	0·480
VI	Rothiemurchus Wood	*Pinus sylvestris*	3·0	3,600	347	0·868
VII	Cressbrookdale Wood	*Fraxinus excelsior*	1·5	900	555	1·388
VIII	Watendlath Wood	*Betula pubescens*	4·0	6,400	106	0·127
IX	Watendlath Grassland	*Pteridium aquilinum*	0·15	9	213	0·533
X	South Bentley Wood	*Quercus petraea*	0·5	100	240	0·600
XI	Cothill Fen	*Schoenus nigricans*	0·5	100	351	0·878
XII	Kingley Vale Wood	*Taxus baccata*	1·5	900	141	0·353

The results do not require much comment. The *Schoenus* tussocks (P. XI) were regularly distributed. The beech trees (Ridley Wood, P. V), bracken fronds (P. IX), and oak saplings (P. X) were distributed at random. The remaining eight populations were aggregated. They were, in order of increasing aggregation, beech trees (Hillock Wood, P. IV), pine trees (P. VI), birch trees (P. VIII), yew trees (P. XII), fruiting heads of *Eriophorum angusti-*

folium (Rannoch Bog, P. III, and Mayo Bog, P. I), ash shoots (P. VII), and finally, flowering heads of *Utricularia intermedia* (P. II).

7. CONCLUSIONS

As will be seen from Tables II and IV, results obtained by the new method, described in this paper, agree closely with those obtained by the methods in current use. The agreement is such that the probabilities of the significance of the deviation of A from unity are of the same order as those for V/M and χ^2. Thus the new method may be used as an alternative to the quadrat methods.

When analysing the type of distribution of trees using the current methods, quadrats have to be marked out on the ground; this is tedious and time consuming. The new method described in this paper is very quick; only distances need to be measured. The line samples used for obtaining random individuals take a little time but are much quicker than the use of quadrats, especially where the number of individuals in each has to be counted. Hence this new method would seem to be a useful substitute for the quadrat methods.

The interest of aggregated populations lies in the reason for and the type of aggregation. The method described above only tells whether or not a population is distributed at random, and how different from random is its distribution. Work is proceeding relating P and I to the density of the individuals and to the distribution patterns.

8. SUMMARY

1. A new method for determining the type of distribution of a population of plant individuals is described.

2. This new method does not require the use of quadrats, but depends on the mean distance between two adjacent individuals, and the mean distance between a random point and its nearest individual.

3. The method has been used on nine synthetic and twelve natural populations. In all cases results were in close agreement with those obtained by current methods.

4. It is suggested that, as it is quicker, the new method might replace the quadrat methods especially in the study of tree distributions where quadrats have to be marked out.

This work was done during the tenure of a Nature Conservancy Research Studentship, and was carried out under the supervision of Professor W. H. Pearsall. For statistical advice thanks are due to Mr. J. G. Skellam who is, however, not responsible for any of the above statements, but has kindly contributed an appendix. Thanks are also due to several people who have assisted with the fieldwork.

TABLE IV

Analysis of Natural Populations

Quadrat size (grid units).	Relative variance						Fit to Poisson			Coefficient of aggregation		
	V/M	t	p	χ^2	Normal deviate	p	χ^2	d.f.	p	A	n	p
Population I. Mayo Bog (*Eriophorum*)												
1	1·6625	9·36	< 0·001	663	8·183	≪ 0·001	85·5021	3	< 0·001	3·3632	97	≪ 0·001
4	2·9491	13·72	< 0·001	291	10·089	≪ 0·001	38·8340	6	< 0·001			
16	5·1605	14·41	< 0·001	124		< 0·001						
25	8·5501	20·68	< 0·001	128		< 0·001						
100	20·3582	23·71	< 0·001	61		< 0·001						
Population II. Mayo Bog (*Utricularia*)												
1	25·9758	352·77	< 0·001	10,364	115·74	≪ 0·001	101·8206	1	< 0·001	2626·78	20	≪ 0·001
4	25·4126	171·80	< 0·001	2,516	56·900	≪ 0·001	144·7795	2	< 0·001			
16	29·7339	99·53	< 0·001	714		< 0·001						
25	35·4324	94·31	< 0·001	531		< 0·001						
100	24·3300	28·57	0·001–0·01	73		< 0·001						
Population III. Rannoch Bog (*Eriophorum*)												
1	1·8066	11·39	< 0·001	721	9·742	≪ 0·001	33·3673	1	< 0·001	2·1990	19	0·008
4	2·7305	12·18	< 0·001	270	9·202	≪ 0·001	25·7258	2	< 0·001			
16	6·7990	20·09	< 0·001	163		< 0·001						
25	9·4359	23·11	< 0·001	142		< 0·001						
100	13·6117	15·45	< 0·001	41		< 0·001						
Population IV. Hillock Wood (*Fagus*)												
1	1·0891	1·26	0·2–0·3	435	1·265	0·20–0·21	48·9776	3	< 0·001	1·2610*	70	0·085
4	2·7421	12·26	< 0·001	271	9·245	≪ 0·001	16·6490	5	0·001–0·01			
16	5·6964	16·27	< 0·001	137		< 0·001						
25	7·8073	18·65	< 0·001	117		< 0·001						
100	19·9965	23·27	< 0·001	60		< 0·001						
Population V. Ridley Wood (*Fagus*)												
1	1·1278	1·81	0·05–0·10	450	1·769	0·07–0·08	2·2872	2	0·3–0·5	1·0318*	35	0·434
4	0·7313	1·89	0·05–0·10	73	−2·002	0·04–0·05	8·9589	3	0·02–0·05			
16	0·4286	1·98	0·05–0·10	10·3		< 0·01						
25	0·9889	0·03	> 0·9	14·8		0·5–0·7						
100	2·1389	1·39	0·2–0·3	6·4		0·05–0·10						

Population VI. Rothiemurchus Wood (*Pinus*)												
1	1·1961	2·77	0·001–0·01	477	2·656	0·001–0·01	7·2231	2	0·02–0·05	1·3135*	69	0·055
4	1·6505	4·58	< 0·001	163	4·019	< 0·001	27·0631	5	< 0·001			
16	2·0503	3·64	0·001–0·01	49		0·001–0·01						
25	2·1917	3·26	0·001–0·01	33		0·001–0·01						
100	2·8780	2·30	0·1–0·2	9		0·02–0·05						
Population VII. Cressbrookdale Wood (*Fraxinus*)												
1	5·0920	57·80	< 0·001	2,032	35·518	⩽ 0·001	462·8216	4	< 0·001	5·4954*	111	⩽ 0·001
4	14·9513	98·18	< 0·001	1,480	40·370	⩽ 0·001	271·1048	7	< 0·001			
16	49·0464	166·42	< 0·001	1,177		< 0·001						
25	63·1342	170·18	< 0·001	947		< 0·001						
100	161·369	196·41	< 0·001	484		< 0·001						
Population VIII. Watendlath Wood (*Betula*)												
1	2·5755	22·25	< 0·001	1,028	17·112	⩽ 0·001	6·1639	1	0·01–0·02	1·6915*	21	0·044
4	1·4675	3·29	0·001–0·01	145	2·993	0·001–0·01	3·6323	2	0·1–0·2			
16	1·6608	2·29	0·02–0·05	39·9		0·02–0·05						
25	1·8113	2·22	0·02–0·05	27·2		0·02–0·05						
100	2·8550	2·27	0·1–0·2	8·6		0·02–0·05						
Population IX. Watendlath Grassland (*Pteridium*)												
1	1·0261	0·37	0·7–0·8	409	0·370	0·71–0·72	0·2401	2	0·8–0·9	1·1652	42	0·232
4	1·1618	1·14	0·2–0·3	115	1·130	0·25–0·26	1·4213	4	0·8–0·9			
16	0·7629	0·82	0·4–0·5	18·3		0·2–0·3						
25	0·5659	1·19	0·2–0·3	8·5		0·05–0·10						
100	0·3318	0·82	0·4–0·5	1·0		0·1–0·2						
Population X. South Bentley Wood (*Quercus*)												
1	0·9523	0·67	0·5–0·6	380	−0·663	0·50–0·51	0·3271	2	0·8–0·9	0·9286	48	0·348
4	0·7744	1·59	0·1–0·2	77	−1·651	0·09–0·10	4·3273	4	0·3–0·5			
16	1·0590	0·20	0·8–0·9	25·4		0·3–0·5						
25	0·3111	1·89	0·05–0·10	4·7		< 0·01						
100	0·1222	1·08	0·3–0·4	0·4		0·05–0·10						
Population XI. Cothill Fen (*Schoenus*)												
1	0·3799	8·76	< 0·001	152	−10·795	⩽ 0·001	163·1565	3	< 0·001	0·2162	70	⩽ 0·001
4	0·2964	4·95	< 0·001	29	−6·381	⩽ 0·001	42·1685	6	< 0·001			
16	0·2463	2·52	0·01–0·02	5·9		< 0·01						
25	0·2826	1·96	0·05–0·10	4·2		< 0·01						
100	0·3305	0·82	0·4–0·5	1·0		0·1–0·2						
Population XII. Kingley Vale Wood (*Taxus*)												
1	1·0479	0·68	0·4–0·5	418	0·683	0·49–0·50	0·2833	1	0·5–0·7	1·8751	28	0·011
4	1·4471	3·15	0·001–0·01	143	2·876	0·001–0·01	10·2857	3	0·01–0·02			
16	2·4681	5·09	< 0·001	59		< 0·001						
25	2·1711	3·21	0·001–0·01	33		0·001–0·01						
100	3·8487	3·49	0·02–0·05	12		0·001–0·01						

* Distances measured to the nearest 10 cm.

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APPENDIX

By J. G. SKELLAM

(The Nature Conservancy, London)

1. If a very large number of particles are distributed independently of one another in a very large area with the same probability density everywhere, then the number falling in a randomly chosen small sub-region is a Poisson variate with a mean proportional to the area of that sub-region.

If we chose a point at random to serve as a centre of a circle and count the number of particles in that circle, we clearly expect the result to be distributed in repeated trials in the same way as if we had adopted a slightly different procedure and had chosen one of the particles at random to act as centre and had counted the number of other particles in a circle of the same size, for the particles are all scattered independently of one another with uniform probability density over a very large area and are very numerous.

Now draw two concentric circles with radii r_1 and r_2 where $r_1 < r_2$.

The probability that the inner one is empty is $e^{-\lambda r_1^2}$ where λ is a constant. The probability that the annulus between the circles is not empty is $1-e^{-\lambda(r_2^2-r_1^2)}$. The probability that the nearest particle to the centre lies between r_2 and r_1, is

$$e^{-\lambda r_1^2}(1-e^{-\lambda(r_2^2-r_1^2)}).$$

By letting $r_1 \rightarrow r_2 = r$ and calling $r_2-r_1 = dr$, we obtain the probability that the nearest particle to the centre lies at a distance between r and $r+dr$. This is

$$e^{-\lambda r^2}\, 2\lambda r dr.$$

The variate $z = \lambda r^2$ then has the distribution given by

$$f(z)dz = e^{-z}dz. \tag{1}$$

2. We now make use of the following theorems, proofs of which are given for example in Weatherburn (1947). 'A First Course in Mathematical Statistics', chap. 8 (Cambridge University Press).

THEOREM I. If the variate z has the frequency function $f(z) = e^{-z}$, then the variate Z defined as the sum of n independent values each distributed as z has the frequency function

$$h(Z) = e^{-Z}\, Z^{n-1}/\Gamma(n). \tag{2}$$

Here $\Gamma(n) = (n-1)\,(n-2)\,\dots\, 3.\, 2.\, 1.$

THEOREM II. If the independent variables Z_1, Z_2 both have the distribution shown above in (2), then the variate x defined by

$$x = Z_1/(Z_1+Z_2)$$

has the frequency function

$$j(x) = x^{n-1}\,(1-x)^{n-1}/B\,(n, n), \tag{3}$$

where $0 \leqslant x \leqslant 1$ and $B(n, n) = \Gamma(n)\,\Gamma(n)/\Gamma(2n)$.

3. If from n random points the distances to the nearest particle are r_1, r_2, ..., r_n, and if from n random particles the distances to the nearest neighbouring particles are $\rho_1, \rho_2, \dots, \rho_n$, then both $Z_1 = \lambda\sum r^2$ and $Z_2 = \lambda\sum\rho^2$ have the distribution (2) by reasons of relation (1) and Theorem I.

It follows from Theorem II that

$$x = \frac{Z_1}{Z_1+Z_2} = \frac{\sum r^2}{\sum r^2+\sum\rho^2}$$

has the distribution (3).

4. For values of n less than 50 the probability that the random variable x is less than a stated value can be obtained from the tables of the integral of (3) prepared by Karl Pearson ('The Incomplete Beta-Function').

The distribution has mean = 0·5 and variance = $1/[4(2n+1)]$ and rapidly tends to normality as n increases. For values of $n > 50$ it is sufficient to take $X = 2(x-0{\cdot}5)\sqrt{(2n+1)}$ as a normal variate with zero mean and unit variance and to refer to the standard tables of the normal integral.

A New Sphenopsid Cone from Iowa[1]

BY

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With Plates XIII-XIV and two Figures in the Text

ABSTRACT

A new Pennsylvanian cone genus (*Litostrobus iowensis*, n. gen., n. sp.), apparently of sphenophyllalean affinity, is described on the basis of a coal-ball specimen from the Urbandale Mine, Urbandale, Iowa. The cone is small and extremely simple in organization. It consists of superposed whorls of twelve bracts each. The bases of the bracts are fused to form shallow cuplike discs. Six sporangia are produced in each whorl; they are erect, each terminating a short, thick, axillary pedicel. Small spores with highly distinctive ornamentation are present in the sporangia.

Comparison with other previously described sphenopsid fructifications reveals morphological differences that warrant recognition of the Urbandale specimen as a new genus.

INTRODUCTION

THIS paper deals with a new genus of sphenopsid cone, based on a single specimen found in a coal ball collected at the Urbandale Coal Mine, Urbandale, Iowa. Although the specimen is fragmentary and not perfectly preserved, it clearly has several unique structural features and represents an interesting addition to our understanding of the fructifications of the American Pennsylvanian Sphenopsida, which have been investigated with significant results during the past decade by Hoskins and Cross (1943), Andrews and Mamay (1951), Baxter (1950), and Levittan and Barghoorn (1948).

MATERIAL

A single small specimen, representing the terminal portion of a cone (the uppermost three whorls of fertile appendages and the sterile apical portion) was found in a coal ball WCB 437-B, from the Urbandale Mine, Urbandale, Iowa. This material is of Des Moines age; it was among the coal-ball specimens collected and donated to Washington University, St. Louis Mo., by Mr. Frederick O. Thompson of Des Moines, Iowa. The figured slides are in the United States National Museum, the others in the Washington University Palaeobotanical Collection.

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[1] Publication authorized by the Director, U.S. Geological Survey.

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Description

The specimen is the top of a cone, only 6·5 cm. long and 3·5 mm. broad, and is slightly crushed. It consists of an axis bearing whorls of twelve bracts united below to form a disc, but free above and overlapping the next whorl. There are six erect pedicillate sporangia in axillary positions above each disc.

Axis. The cone axis is best shown in certain of the transverse sections. Like the rest of the specimen, it is slightly flattened. In a median internodal section (Pl. XIII, Fig. 7) it measures about 0·80 by 0·35 mm. in diameter and shows an elongate hexagonal outline, the flattened surfaces having been produced by appression of the axis with the six sporangia in each whorl.

Although the outline of the cone axis is distinct in many of the sections, the cellular details cannot be satisfactorily distinguished. There is indication of a distinct epidermis consisting of a single layer of rather small cells; within this layer, cell size appears to increase gradually towards the centre of the axis. In one transverse section, two groups of small cells with rather thicker walls might represent part of the xylem, which I imagine to have been triarch. Longitudinal sections merely show some narrow elongated cells.

The bracts. The bracts are laterally fused into a continuous disc where they depart from the cone axis; the lines of fusion are indistinguishable. The disc departs from the cone axis at a gently ascending angle, forming a shallow cuplike structure (Pl. XIV, Figs. 13, 14). Farther out it assumes a more strongly ascending position before it dissects into discrete bracts.

Various stages in this dissection may be seen in the series of ascending sections shown in Pl. XIII, Figs. 1, 2, 3, 4, and 7. In Figs. 1 and 2, which show the two lowermost sections, the still undissected disc appears as a continuous ring of tissue surrounding the remains of the cone axis and the bases of the sporangial pedicels. In Fig. 3 one bract has been separated from the disc, and in Fig. 4 another bract. In the most terminal section in this series, shown in Fig. 7, the cone is seen to be surrounded by the recognizable remains of eight free bracts; in the lower left part of the photograph a still undissected sector of the disc appears to be in the process of dividing into at least three, and possibly four, additional bracts. Thus it seems certain that the total complement of a sterile whorl was twelve bracts; the distinct arrangement of bracts, two to a sporangium in sections that are less completely preserved than the section shown in Fig. 7, but that contain as many as ten recognizable bracts in a whorl, further supports this conclusion.

Most of the bracts are rather crushed and distorted, owing to the delicate nature of the tissue that made up their greater bulk (Pl. XIII, Fig. 6). The less distorted bracts, however, which show thick fusiform transverse-sectional outlines when first separated from the disc, measure about 0·85 mm. in width and 0·28 mm. in thickness. They are delimited by a single layer of epidermal cells; these cells have thickened walls and most of them have dark brown contents that contrast the epidermis with the inner tissues of the bract. The inner tissues consist of very thin-walled parenchymatous cells somewhat larger than the epidermal cells.

The epidermal cells, when sectioned parallel to the outer surface of the bracts, show sinuous interlocking outlines (Pl. XIII, Fig. 5), as in *Bowmanites bifurcatus*, *B. römeri*, and *Peltastrobus reedae*; Walton's observation (1940) that this distinctive type of epidermal cell is present in the leaves of certain species of *Sphenophyllum* has been interpreted as evidence in support of a sphenophyllalean alliance for both *Bowmanites römeri* and *Peltastrobus reedae*, two fructifications that have few other points of similarity.

Each bract contains a single small vascular strand that occupies a central position within the soft parenchyma. The strand consists of only four or five narrow tracheides, each tracheide about 25 μ in diameter (Pl. XIII, Fig. 6). Further details of the organization of these strands cannot be distinguished because of imperfect preservation.

The ascending tips of the bracts overlap the next superior whorl, as may be seen in longitudinal section in Pl. XIV, Fig. 14; here the bract on the left side of the cone ascends to a position well towards the tip of the sporangium above it in the next whorl. The vertically ascending extent of this particular bract is about 3 mm., which likely represents an average length. In nearly every transverse section two concentric whorls may be seen, the outermost composed of the overlapping bracts of the lower whorl (see Pl. XIV, Fig. 7).

The apex of the cone is preserved, but not in sufficient detail for complete description (Pl. XIV, Figs. 13, 14). It consists of the apical meristem, enveloped by the small, immature sterile appendages of the first two whorls. About 0·25 mm. below the lower of these two whorls arises the third whorl, which is also sterile. The next whorl below is fertile; the internode between the third and fourth whorls is about 1·8 mm. long. Although the length of internodes below this one cannot be accurately established from the sections on hand, the known dimensions of sporangia suggest that it was about 2·5 mm.

Sporangia. The fertile members of each whorl are extremely simple in organization. The sporangia are erect (orthotropous) and are produced singly at the tips of short, stout pedicels. The pedicels arise from distinctly axillary positions, i.e. from points of divergence of the sterile disc and the cone axis.

The transverse series of sections provides the clearest picture of these important organizational details. The section illustrated in Pl. XIII, Fig. 1, has been cut below the concavity in the upper surface of the sterile disc, so that the fleshy portion of the disc appears as a continuous plate of tissue. Also shown here are three of the pedicels of this whorl, each about 0·2 mm. in diameter. The significance of this section lies in its demonstration of an organic continuity of the tissues of the pedicels with those of the cone axis on one side and those of the sterile disc on the other. This continuity clearly illustrates the axillary origin of the pedicels. The pedicels may be viewed longitudinally in Pl. XIV, Fig. 13.

Higher sections in this series illustrate the appearance of three additional pedicels, bringing the total to six, the full complement of a fertile whorl.

Diameters of the pedicels increase to about 0·4 mm., and, in better-preserved specimens, a small central vascular strand can be seen (Pl. XIII, Figs. 3, 4). As the sections proceed higher and intercept the concavity in the sterile disc, the tissues of the inner surface of the disc protrude upward into the spaces between pedicels (Pl. XIII, Fig. 2). We may thus visualize the sterile disc as a shallow cup with a coarsely fluted inner surface, the ridges radiating outward from a common centre at the bottom, and one pedicel lying in each of the resultant grooves.

Proceeding still farther in the distal direction, the diameters of the pedicels increase to nearly 1 mm. This increase in size is accompanied by a marked differentiation in the histological details of the pedicels. At their bases the pedicels are composed of a thin epidermis and a compact inner tissue of small parenchymatous cells. As the base of the sporangium is approached, however, a marked differentiation of tissues takes place; while the adaxial tissues of the pedicels remain relatively unchanged, the cells of those tissues in the abaxial portions increase in size (Pl. XIII, Figs. 2, 3). Cells of the abaxial surface of the epidermis become especially large and slightly indurated. These cells form the outer layer of the sporangial wall, as is shown in slightly higher sections that intercept the sporangial cavities.

The longitudinal aspect of the sporangia and pedicels is shown in Pl. XIV, Figs. 13 and 14. In Fig. 13 one may clearly see the axillary point of origin of the pedicel on the left and the orthotropous position of its sporangium. The delicate inner tissue of the pedicel continues upward to form a parenchymatous lining to the sporangial cavity; the distance from the point of origin of this pedicel to the base of the sporangial cavity is 0·4 mm. A tiny vascular strand of narrow annular tracheides may be seen under higher magnification; it extends upward through the pedicel, nearly reaching the base of the sporangial cavity.

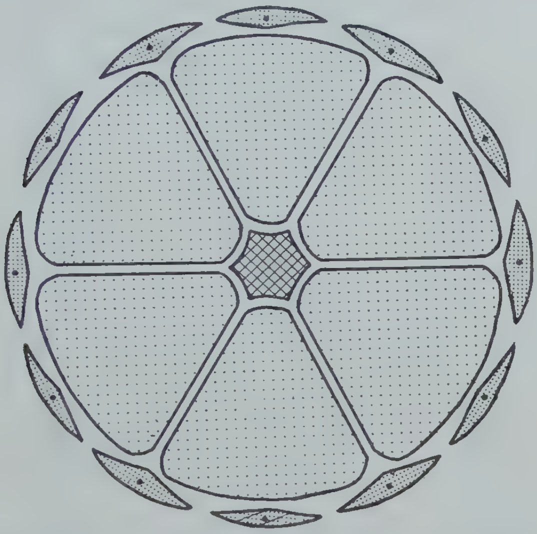
The cells of the outer layer of the sporangial wall are elongate in the direction paralleling the cone axis. They are largest on the abaxial surface of the sporangium and gradually decrease in size towards the cone axis. The large cells measure about 150μ in length, 40μ in width, and 40μ in thickness. A slight sinuosity in the outlines of these cells has been noted; however, it is not as marked as that shown by the cells in the outer epidermis of the bracts.

The inner parenchymatous tissue of the sporangial wall is several layers thick, and the component cells are much smaller and more delicate than those of the outer layer. This tissue completely lines the sporangial cavity; its remnants may be seen in nearly every section of the specimen.

Each of the two whorls studied in transverse section contains six sporangia (see Pl. XIV, Fig. 8). A median transverse section through an internode shows the six sporangia of a whorl closely appressed to each other; the consequent flattening of their lateral walls gives the sporangia a roughly triangular outline. The greatest transverse dimensions of the sporangia average about 1·3 mm.

Seen in longitudinal section (Pl. XIV, Fig. 13), the sporangia display more or less elliptical outlines. The complete sporangium shown at the left of this figure measures 2 mm. from its tip to the base of the pedicel. Although the basal parts of the sporangia in the next lower whorl do not appear in this section, the dimensions of their upper parts are somewhat larger than comparable measurements of the complete sporangia at the upper part of the figure; it is estimated that their total lengths were probably closer to 3 mm.

Vascular system. Little is known about the vascular organization of the cone. The number of axial strands cannot be determined; each bract and each sporangial pedicel contains a single small vascular strand, but how these arise from the axial system with relation to each other cannot be stated.

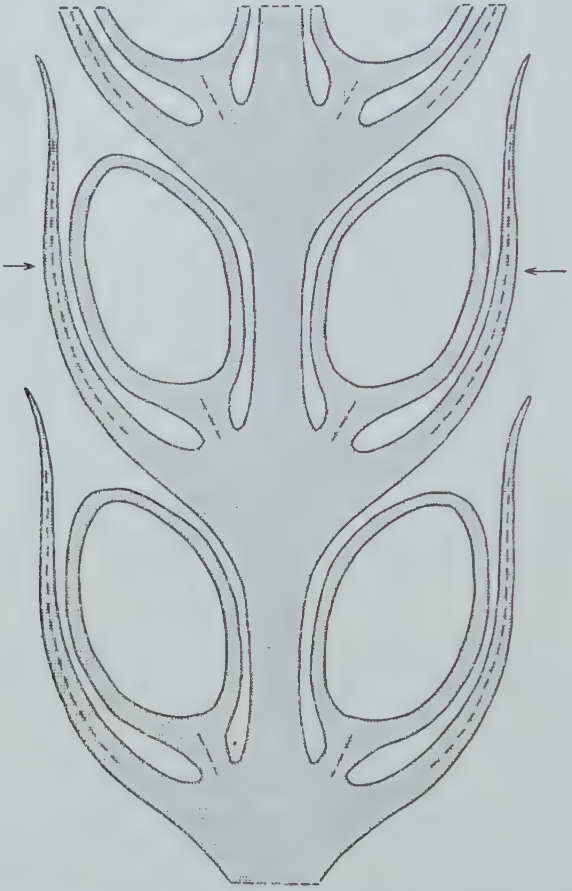


TEXT-FIG 1. Transverse sectional diagram of *Litostrobus iowensis*, representing the plane indicated by arrows in Text-fig. 2. Note the position of the bracts in relation to the sporangia. Cross hatching = cone axis; coarse stippling = sporangia; fine stippling = bracts; large black dot within each bract = vascular strand.

The series of transverse sections illustrated in Pls. XIII-XIV, Figs. 1, 2, 3, 4, and 7, yields certain information regarding the vascular system of the sterile disc that aids in interpreting the manner of division of the disc and the orientation of the resultant free bracts with reference to the sporangia.

In the lowest section of this series (Pl. XIII, Fig. 1) the sterile disc has been sectioned below its points of dissection and appears as a continuous ring of tissue. Several vascular strands may be seen in the disc. These strands may be followed upward in the series, each strand eventually passing out into one of the free bracts. Although no single section shows more than five or six of the strands, it is quite clear, from their positional relationships with the sporangia, that the strands are twice as numerous as the sporangia. The strands are situated one directly *opposite* each sporangium and the others *alternate* with the sporangia. This point is further illustrated in Pl. XIII, Fig. 3; here one bract has been separated from the disc, and its position is clearly *opposite* a sporangium. These facts support the conclusions, stated previously, that there are twelve bracts composing each sterile disc.

The presence of two bracts to each sporangium raises a morphological question that may be interpreted in various ways; the one favoured is that the disc consists of *twelve* independent bracts, *every other* one of which is fertile. If it were composed of six forked bracts, the sporangium should not be axillary to one side of the fork.



TEXT-FIG. 2. Diagrammatic longitudinal section of *Litostrobus iowensis*. Arrows indicate the transverse plane represented in Text-fig. 1. Broken lines within bracts and pedicels indicate the known extent of the vascular strands.

Spores. The sporangia were preserved prior to dehiscence; hence their contained spore masses are complete and well preserved. With the exception of those contained in two of the sporangia in the uppermost fertile whorl, the spores appear to have matured prior to preservation. The immature spores are largely collapsed, and their extremely thin walls are devoid of the sculpturing that characterizes the more mature ones.

The mature spores are spherical. Variation in spore diameters is considerable, ranging from 65 to 100μ. The spore walls apparently consist of two membranes, a thick exospore and a thin, delicate endospore. The endospore is generally shrunken and collapsed, appearing as an irregular-shaped mass within the spore cavity. Some endospores present a simulated triradiate appearance (Pl. XIV, Fig. 10).

The exospore is characterized by distinctive reticulate ornamentation. The ridges show very little relief; they delimit irregular-shaped areas averaging about 6μ in their greatest diameters. In many of the spores the stronger development of some of the ridges produces a coarse secondary reticulation (Pl. XIV, Figs. 11, 12).

Some spores exhibit triradiate ridges; the rays of these ridges reach about 20μ in length. The reticulations of the exine continue through the areas between the rays. A peculiar feature of the spores of this cone is the apparently continuous circular groove surrounding the proximal surface of the spore; in spores that have the triradiate ridge, this groove closely encircles the area delimited by the ridge (Pl. XIV, Fig. 9). In some spores the spore wall has been partly torn along this circular groove. This tear, and the apparent absence of a triradiate ridge in some of the spores, suggests that this circular groove may have been an actual dehiscence mechanism, the proximal part of the spore membrane being shed as an operculum-like cap.

There is no evidence of heterospory.

DISCUSSION

The verticillate organization and fused sterile appendages of this cone broadly limit its possible affinities. They immediately exclude the possibility of a lycopsid alliance and place the specimen within the Sphenopsida.

The presence of six sporangia in each fertile whorl and apparently twelve bracts in each subtending whorl strongly suggests a trimerous basic symmetry, and corollarially a sphenophyllalean affinity for the specimen. The significance of the sinuous outlines of the epidermal cells of the bracts in suggesting sphenophyllalean affinities has been mentioned above.

Closest comparisons of the Urbandale specimen may be made with the sphenophyllalean genus *Bowmanites*. As revised by Hoskins and Cross, *Bowmanites* has nineteen species, of which seven are known as petrifactions. However, all these seven differ from *Litostrobus* in having inverted sporangia borne by sporangiophores on the upper surfaces of the bracts. Another difference is that there are apparently twelve distinct bract units rather than six forked ones, as in *Bowmanites bifurcatus*.

Certain species of *Bowmanites*, based on compressed specimens, have been described as having axillary sporangia (*B. verticillatus*, *B. angustifolius*, *B. tenerrimus*), and *B. trichomatosus* apparently has nearly sessile sporangia. In none of these species is there convincing evidence of inverted or anatropous sporangia. Thus these species have certain of the features whose presence in the Urbandale specimen distinguishes this specimen, in a striking manner, from petrified *Bowmanites* species. However, Hoskins and Cross, in their monograph of the genus, have written: '. . . Usually considered to be sessile, but better material may show sporangiophores present, a characteristic of the genus' (*B. trichomatosus*, p. 143); '(Possibly in older specimens the sporangiophore as well as the bract elongated and thus the sporangium itself is not borne in an axillary position, but, rather, distally upon the pedicel)' (*B. angustifolius*, p. 145). These statements illustrate some of the difficulties encountered in attempting to establish relationships between well-preserved petrified specimens and compressed specimens whose internal structural features have been obscured by compaction; they also indicate a predilection on the parts of the monographers to discount any possibility of bowmanitean affinity for cones whose sporangia are axillary and lacking in distinct sporangiophores.

The notable simplicity of the Urbandale specimen in contrast with the complex organization of bowmanitean fructifications, as they are known from petrified material, appears to warrant a new generic name; indeed, the specimen is as distinct from *Bowmanites* as is *Sphenostrobus*. Its sphenophyllalean nature seems fairly clear, but to assign it to *Bowmanites* on the basis of possible similarities with the poorly understood compressed specimens mentioned above seems far less justifiable than to distinguish it from that genus on the basis of actual differences from the well-known petrified specimens.

Its orthotropous sporangia and the more general features of its spores make the Urbandale specimen superficially resemble *Sphenostrobus Thompsonii*. However, *Sphenostrobus* has sessile sporangia, borne in whorls of sixteen, each with a single alternating bract; furthermore, the stelar organization is tetrarch, a fact that was interpreted by Levittan and Barghoorn as a strong indication of noeggerathialean, rather than sphenophyllalean affinity. These features clearly distinguish *Sphenostrobus* from the Urbandale specimen as well as from *Bowmanites*.

The generic name *Litostrobus* (Greek *litos*; plain, simple) is proposed for the Urbandale specimen, with reference to the structural simplicity of the cone. Its distinguishing features, in so far as they are presently known, are summarized in the following diagnoses:

Litostrobus iowensis, n. gen., n. sp.

Generic diagnosis. Cone verticillate, the appendages produced in multiples of three. Whorls superposed, each consisting of basally fused bracts and axillary sporangia. Bracts twice as numerous as sporangia, arranged one opposite a sporangium, one alternate. Sporangia orthotropous, each terminating a short axillary pedicel. Heterospory not known.

Specific diagnosis. Cone small, greatest known diameter about 3·5 mm.; nodes about 2 mm. apart. Each sterile disc marginally divided into twelve free ascending bracts, each bract containing a single vascular strand. Epidermal cells of bracts with sinuous interlocking outlines, and filled with brown opaque mucilaginous (?) substance.

Sporangia borne six in a whorl; sporangia ovoid, to 3 mm. long, 1·3 mm. in diameter. Sporangial walls several cells thick, the outer epidermal layer slightly indurated, the inner layer consisting of several thicknesses of delicate parenchymatous cells.

Pedicels to 0·4 mm. long, each containing a narrow vascular strand that extends nearly to the base of the sporangial cavity.

Spores spherical, ranging from 65 to 100μ in diameter. Perisporium absent. Exosporal membrane with a fine reticulation of ridges having faint relief; some ridges more pronounced than others, resulting in a coarse secondary reticulation. Where triradiate ridges present, rays to 20μ long. A circular groove on the proximal surface, clearly circumscribing the triradiate ridge where the latter is present.

The remarkable simplicity of *Litostrobus* invites speculation regarding its evolutionary position among the sphenophyllalean fructifications discovered thus far.

Arnold (1947, p. 138) has written: 'The trend in the Sphenophyllales appears to have been toward strobilar complexity, and unlike the lycopods there is no indication of seed production.' To interpret *Litostrobus* in the light of this statement would lead to the conclusion that it is a primitive, unelaborated form of fructification.

However, a consideration of the stratigraphic relationships of those sphenophyllalean fructifications whose structural details are best known would seem to indicate a broad trend in the opposite direction, i.e. towards strobilar simplicity, by progressive reduction and sterilization of parts. It may be generally stated that the most complex forms are the oldest, whereas the simpler forms are found higher in the geologic column. *Cheirostrobus pettycurensis*, for example, the most complex of all pteridophytic fructifications, occurs only in the Pettycur limestone of Early Carboniferous age, in Scotland; *Bowmanites fertilis*, a relatively complex form, occurs only in English Lower Coal Measures, and rocks of comparable age in Europe. *Litostrobus*, the simplest form, occurs in the Des Moines group; *Bowmanites bifurcatus*, a relatively simple form, occurs in the McLeansboro formation.

Although an absolute correlation between the geologic age and structural complexity of the various other sphenophyllalean fructifications intermediate between the extreme forms cannot be demonstrated, the wide stratigraphic separation between the extreme forms seems to suggest a general evolutionary trend towards reduction rather than proliferation. Accordingly *Litostrobus* may best be interpreted as a highly advanced and reduced fructification, rather than a primitive one.

SUMMARY

1. A single cone specimen was found in a coal ball from Urbandale, Iowa. The specimen is extremely simple in organization, and certain of its features strongly favour its assignment to the Sphenophyllales.

2. On the basis of comparison with other sphenophyllalean cones the specimen is recognized as a new genus, and the binomial *Litostrobus iowensis* is proposed.

3. The simplicity of the cone is interpreted as the result of reduction, and hence indicative of an advanced condition rather than a primitive one.

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EXPLANATION OF PLATES XIII AND XIV

Illustrating Sergius H. Mamay's paper on 'A New Sphenopsid Cone from Iowa'

(*a* = axis; *b* = bract; *d* = disc; *p* = pedicel; *v* = vascular strand.)

PLATE XIII

Fig. 1. A transverse section through the basal, undissected portion of a disc, showing three pedicels in their axillary positions. Several vascular strands are shown in the tissues of the disc; also shown are several bracts and one sporangium from the whorl below. Slide T–28. (×19.)

Fig. 2. A transverse section slightly higher than that shown in Fig. 1. Note the increased diameters of the pedicels, and the positional relationships between the pedicels and the vascular strands of the disc, several of which are shown. Slide T–30. (×19.)

Fig. 3. A transverse section slightly higher than that shown in Fig. 2. A sixth pedicel is shown at the left of the axis. Dissection of the disc has begun, the first bract having been separated at the bottom of the photograph. The tips of bracts from the whorl below may still be seen. Slide T–35. (×19.)

Fig. 4. A transverse section slightly higher than that shown in Fig. 3. A vascular strand is shown in the uppermost pedicel. The section has intercepted the bottoms of the spore cavities in two sporangia. Slide T–36. (×24.)

Fig. 5. Epidermal cells of a bract, showing sinuous outlines and dark contents. Slide L–22. (×135.)

Fig. 6. Two bracts, transversely sectioned. Note the dark contents of the epidermal cells and the small vascular strand in each bract. Slide T–22. (×40.)

PLATE XIV

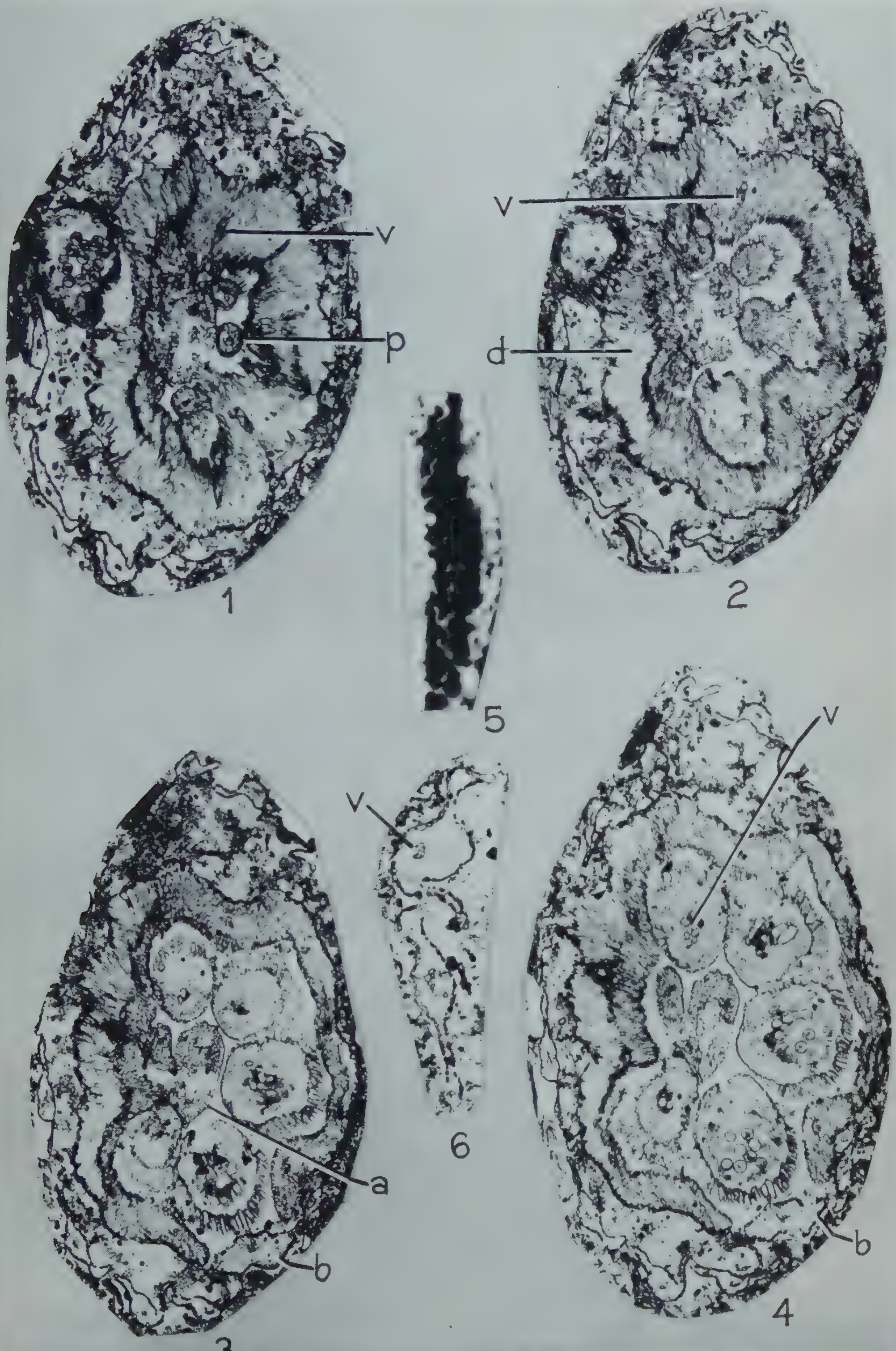
Fig. 7. A transverse section slightly higher than that shown in Fig. 4. Note the nearly completed dissection of the disc, and the positional relationships between bracts and sporangia. Slide T–41. (×22.)

Fig. 8. A transverse section from about the middle of the internode below the whorl illustrated in Figs. 1, 2, 3, 4, and 7. Note the similarity to a transverse section of a marattiaceous synangium. Slide T–11. (×24.)

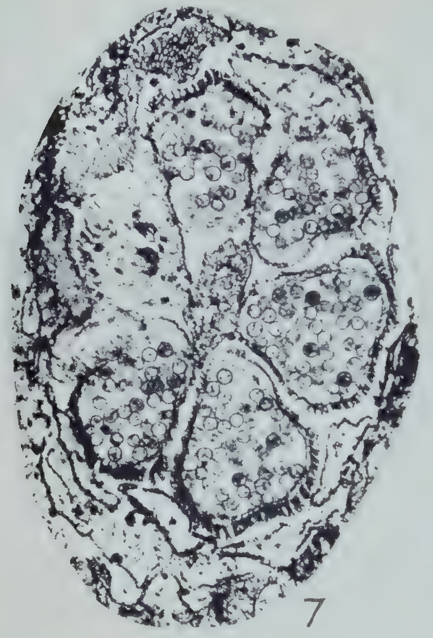
Figs. 9–12. Spores, illustrating variation in size. All figures ×312. Fig. 9 (slide T–15) shows the triradiate ridge and the circular groove surrounding it. Fig. 10 (slide T–15) shows the trilete appearance of the delicate, shrunken endosporal membrane. Figs. 11 (slide L–23) and 12 (slide L–11) show the characteristic reticulate ornamentation of the exospore.

Fig. 13. A nearly median, slightly oblique longitudinal section, showing the sterile apical portion of the cone and parts of two fertile whorls. Note cuplike shape of the disc, and the axillary attachment of the pedicel of the sporangium at the left of the upper fertile whorl. Slide L–8. (× 15.)

Fig. 14. A slightly oblique tangential section. Note the extent to which the bract at the left overlaps the succeeding whorl. Slide L–5. (× 15.)



S. H. MAMAY—PLATE I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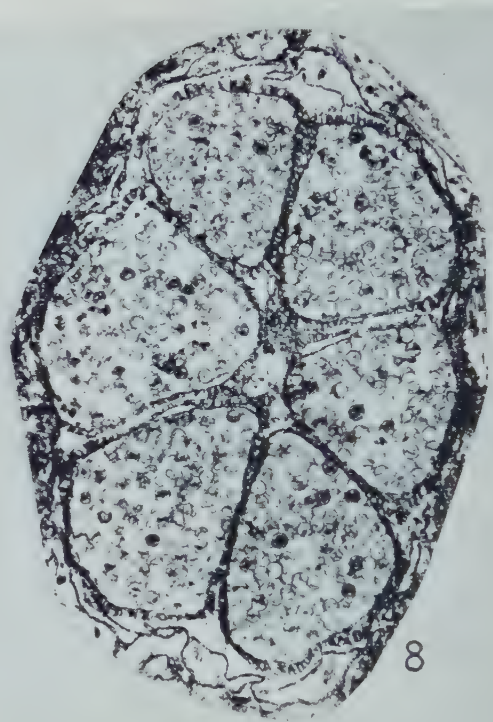
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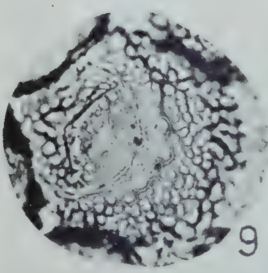
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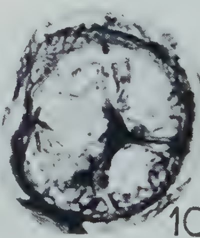
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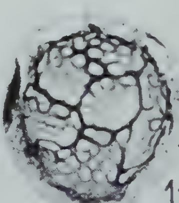
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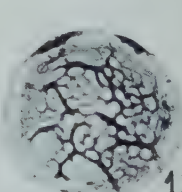
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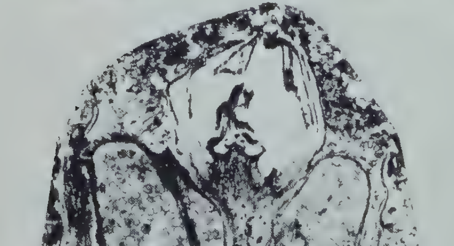
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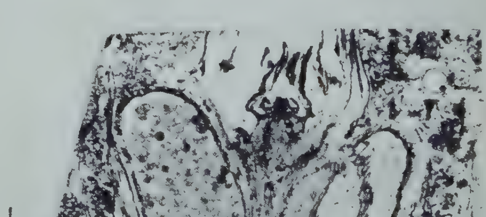

14

S. H. MAMAY—PLATE II

The Function of the Hilum in Some Papilionaceae in Relation to the Ripening of the Seed and the Permeability of the Testa

BY

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With nine Figures in the Text

ABSTRACT

In seeds of *Trifolium repens*, *T. pratense*, and *Lupinus arboreus*, the hilum is a hygroscopically activated valve in the impermeable epidermis of the testa. When relative humidity was low the fissure in the hilum opened permitting the seed to dry out; when the relative humidity was high the fissure closed obstructing the absorption of moisture. During seed-ripening the moisture content fell readily to approximately 25 per cent., and thereafter more slowly until the epidermis became impermeable at approximately 14 per cent. moisture content. Further drying of the seed took place only by diffusion of water vapour through the hilum. 'Hard' seeds tended to have a moisture content in equilibrium with the lowest relative humidity to which they had been exposed. They absorbed moisture under conditions of gradually increasing relative humidity such that the hilar fissure remained open. The duration of the impermeable condition increased with the degree of desiccation brought about by loss of water through the hilum.

INTRODUCTION

IT is characteristic of the ripe seeds of most leguminous plants that the testa strongly resists the penetration of water. Because of their impermeability the seeds may remain dormant in the soil for many years. This property is of ecological importance and it gives rise to practical problems in the propagation of some economic plants. Among agronomists seeds which fail to imbibe water within a standard testing period are termed 'hard' seeds and are held to be of much less value than seeds which germinate promptly.

The literature dealing with the anatomy and function of the testa of leguminous plants has been reviewed by Hamly (6), Zimmerman (14), Watson (12), and Porter (10).

There has been disagreement as to the location of the impermeable layer in the testa. Among recent workers Watson (12) and Zimmerman (14) have regarded the cuticle as the impermeable layer, whereas Coe and Martin (1) concluded that the cuticle was permeable and that the portions of the epidermal cells in the region of the light line constituted the impermeable layer.

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Raleigh (11) held that shrinkage of the testa during ripening played an essential part in the development of the impermeable condition.

Different conclusions have been arrived at regarding the influence of atmospheric humidity on hard seeds. Guppy (5) concluded that such seeds were 'non-hygroscopic'. Ewart (4) observed that some leguminous seeds which were incapable of imbibing water nevertheless gave up water when stored in a dry atmosphere. Kuehn (8) showed that artificial drying may increase the percentage of hard seeds and prolong dormancy. Esdorn (3) found that percentage of hard seeds fluctuated in accordance with seasonal changes in humidity.

Few observations have been made on the relationship between the water content of seeds and the permeability of the testa. Jones (7) found that in samples of *Vicia villosa* seed impermeability was rarely encountered when the water content exceeded 14 per cent. Guppy (5) observed that the moisture content of the impermeable seeds of many different species was far below normal hygroscopic equilibrium level.

Previous workers have regarded the hilum as having no significance in relation to the impermeability of the testa. Mattirolo and Buscalioni (9) studied the hilum and observed the hygroscopic movements which result in the opening and closing of the median fissure, but they concluded that the function of the hilum was to facilitate the emergence of the radicle at germination. Corner (2) suggested that the hilar fissure provides a passage for the exchange of gases involved in respiration during seed development and germination.

No observations have been recorded which explain the simultaneous occurrence in the ripening seed of two apparently incompatible processes, namely, the development of an impermeable testa and the desiccation of the embryo. A study of the ripening seeds of different leguminous plants was therefore undertaken to find an answer to this question. The present report records observations on seeds of some Papilionaceae in which special attention was paid to the function of the peculiar hilar mechanism.

MATERIAL AND METHODS

Seeds of the following species were studied: *Trifolium repens L.* white clover, *Trifolium pratense L.* red clover, and *Lupinus arboreus L.* tree lupin.

For some experiments it was necessary to have seed ripened at specific levels of relative humidity. This material was obtained by picking seed heads which were commencing to ripen and mixing them with ten times their volume of ryegrass seed, the moisture content of which had been adjusted so that the relative humidity of the interstitial air was at the required level. The ryegrass seed was changed daily for the first 4 days and thereafter twice weekly for 2 weeks. The clover seed was finally rubbed out by hand and stored in a desiccator at controlled humidity. By this method seed could be ripened quickly at 70 per cent. relative humidity without injury from moulds.

For storage at controlled humidity desiccators were used containing sulphuric acid of known density. The relative vapour pressures of sulphuric acid solutions of different density were taken from tables published by Wilson (13).

All sorption experiments and germination tests were carried out at 20° C.

Samples consisting solely of hard seeds were obtained by steeping seed in water at 20° C. for 8 days. All seeds which imbibed water were then discarded. The hard seeds remaining were dried for one minute between sheets of filter paper and placed in a desiccator at controlled humidity.

When permeable clover seed was required samples were scarified by rubbing the seeds between sheets of medium grade emery paper.

The moisture content of clover seed was determined by drying at 105° for 24 hours. Lupin seeds were cut in two and dried for 24 hours. Moisture content is recorded throughout as the percentage of dry weight.

For anatomical studies 10 μ sections of imbibed seeds embedded in 10 per cent. gelatine were cut with a freezing microtome and stained with safranin. Illustrations were drawn with the aid of a camera lucida.

STRUCTURE OF THE HILUM

The anatomy of the testa in the region of the hilum is essentially the same in the three species studied and therefore a description of only one species, tree lupin, is given. The structure is illustrated in Figs. 1 and 2.

The hilum of the tree lupin seed is an oval-shaped scar about 1 mm. long and 0·6 mm. broad lying in a depression. Tissue derived from the funiculus overlies the epidermis of the testa. In contact with the cuticle of the epidermis is the counter-palisade layer composed of cells elongated in the direction perpendicular to the surface of the seed and having walls heavily thickened except at the outer ends. Overlying the counter-palisade layer is parenchyma with large intercellular spaces. This tissue is much disorganized near the place of abscission.

In the long axis of the hilum there is a groove in the funicular tissue coinciding with a fissure in the epidermis of the testa. Immediately below the fissure lies the tracheid bar or vascular isle, a band of tissue composed of short tracheids with large pits. Surrounding the tracheid bar is tissue composed of stellate cells. The intercellular spaces in this tissue are continuous with those of the subepidermal layer of hour-glass or pillar cells which underlies the epidermis everywhere except at the hilum and the strophiole.

EXPERIMENTAL

Hygroscopic movement of the hilum: In ripe seeds the epidermis of the testa is split along the median groove of the hilum. This fissure opens and closes in response to changes in the relative humidity of the external atmosphere. Movement of the hilum of tree lupin seeds was observed under the microscope at a magnification of 100×. Seeds were placed in a small glass cell and enclosed with a microscope cover slip. When a piece of anhydrous calcium chloride was introduced into the cell the fissure opened within one minute.

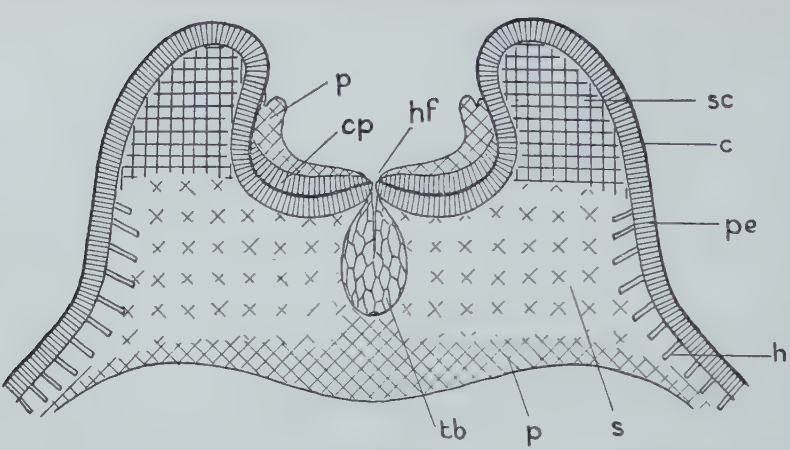

FIG. 1. Diagrammatic illustration of the tree lupin seed showing a transmedian section of the hilum and adjacent tissues. *p*, parenchyma; *cp*, counter-palisade; *hf*, hilar fissure; *sc*, sclerenchyma; *c*, cuticle; *pe*, palisade epidermis; *h*, sub-epidermal layer of 'hour glass' or 'pillar' cells; *s*, stellate cells; *tb*, tracheid bar.

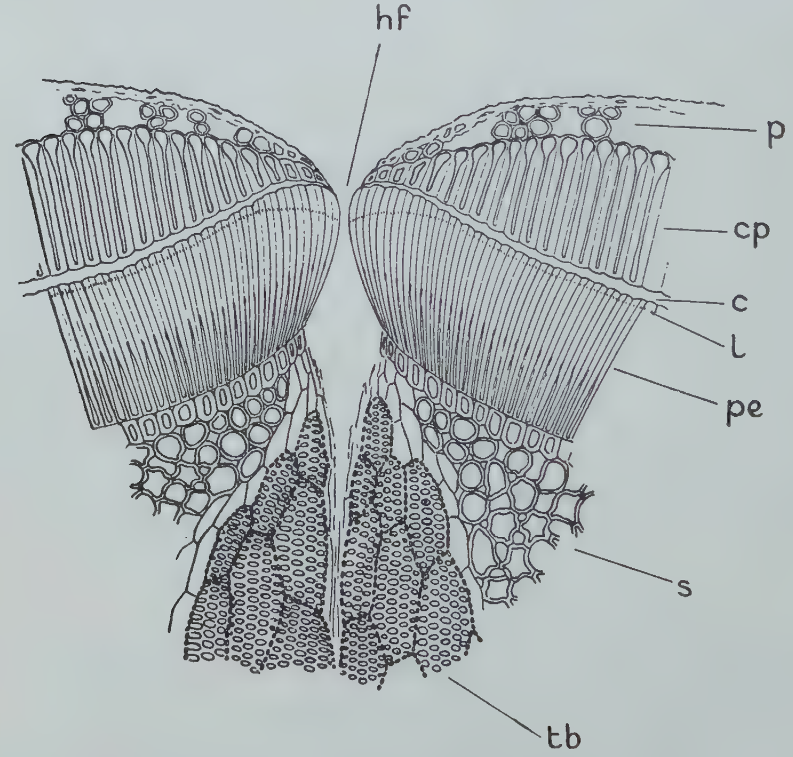

FIG. 2. Transmedian section of the hilum of tree lupin. *hf*, hilar fissure; *p*, parenchyma; *cp*, counter-palisade; *c*, cuticle; *l*, light line; *pe*, palisade epidermis; *s*, stellate cells; *tb*, tracheid bar.

The breadth of the gap varied in 8 seeds from 15 to 75 μ being greatest in seeds of high moisture content. When the calcium chloride in the cell was replaced by a piece of damp filter paper the fissure closed within one minute. These reactions suggest that the counter palisade functions as a motor tissue. When the relative humidity falls the counter palisade dries and shrinks drawing the margins of the fissure upwards and apart. When the relative humidity rises the counter palisade swells causing the fissure to close.

The opening of the hilar fissure was further demonstrated by the diffusion of iodine vapour into the seed. Hard seeds of tree lupin, red clover, and white clover were placed in a desiccator containing calcium chloride and also a small dish of iodine. After 3 hours at 30° C. the seeds were removed and bisected through the hilum. Microscopic examination showed that in every seed the sub-hilar tissue, especially the tracheid bar, was stained brown. The experiment was repeated after replacing the calcium chloride in the desiccator with a small piece of damp filter paper. Under these conditions the counter palisade and parenchyma on the surface of the hilum stained but even after 24 hours no iodine vapour had penetrated the seed.

Sorptive capacity of the seeds: Preparatory to a study of the hygroscopic properties of intact seeds the sorptive capacity of the seed substance was determined. Seeds were coarsely ground and 2 g. samples of the meal were stored in desiccators at different levels of relative humidity. After 10 weeks when periodical weighing indicated that the seed material was in hygroscopic equilibrium with the sulphuric acid solutions the moisture content of each sample was determined. To ascertain the maximum hysteresis in one species, white clover, the determinations were repeated with samples which had previously been dried over concentrated sulphuric acid. The results are shown in Fig. 3.

Sorption studies with hard seeds and scarified seeds:

Experiment 1

Two samples, each of approximately 1 g. were taken from a quantity of impermeable white clover seeds ripened at 70 per cent. relative humidity. One of the samples was scarified. Both lots of seed were then held at 70 per cent. relative humidity for 4 days and weighed periodically. Thereafter they were transferred successively to desiccators at 30, 70, 30, 0, 70, and 0 per cent. relative humidity. The samples remained for 7 days in each desiccator and were weighed periodically. Finally, the moisture content of the seed was determined and the moisture content at each periodical weighing calculated. The results are shown in Fig. 4.

Reference to the sorption curves in Fig. 3 indicates that the scarified seed approached hygroscopic equilibrium under both dry and humid atmospheric conditions. The hard seeds also tended to come to equilibrium when the atmosphere was drier than any to which the seed had previously been exposed. But when hard seeds were transferred from a dry atmosphere to one more humid there was little re-sorption of water. In the first day the moisture content increased by 0·3–0·6 per cent., but during the remainder of the storage period no perceptible increase occurred. Scarified seed displayed normal hygroscopic reactions, whereas in hard seeds desorption proceeded normally but absorption was obstructed.

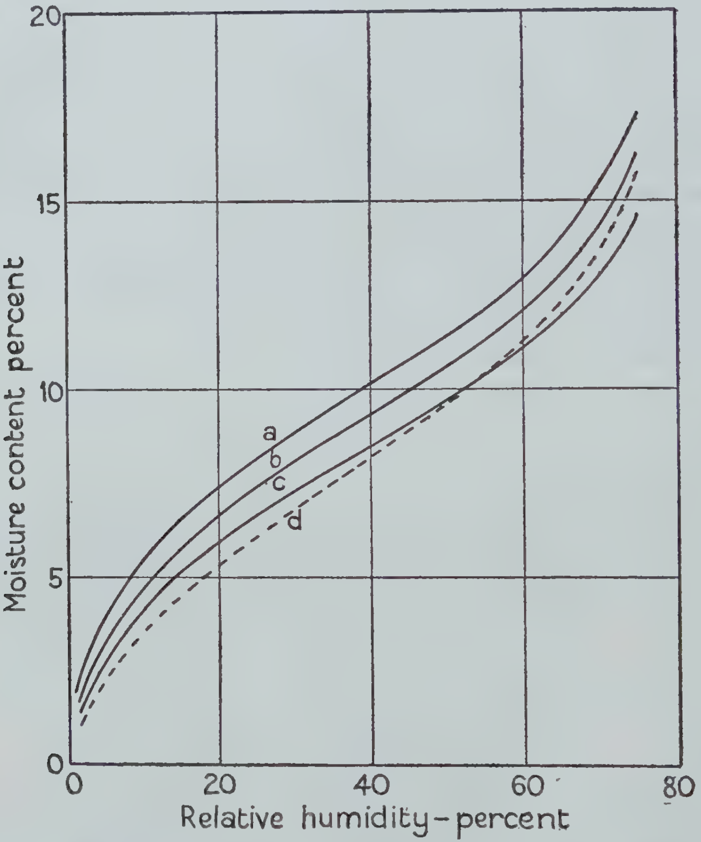

Fig. 3. Relationship between relative humidity and moisture content of seeds in hygroscopic equilibrium with the atmosphere at 20° C. *a*, tree lupin; *b*, white clover; *c*, red clover; *d*, white clover previously dried over concentrated sulphuric acid.

Experiment 2

Samples of hard seeds of white clover and red clover both ripened at 65 per cent. relative humidity were treated in the following ways. Some seed of each species was scarified and 2 g. samples of untreated and scarified seed were weighed out. In addition approximately 0·4 g. of impermeable red clover seeds were taken and the hilum of each seed covered with petroleum jelly.

The samples were placed together in a desiccator at 65 per cent. relative humidity and then transferred successively to desiccators at 50, 33, 70, 0, and 70 per cent. relative humidity. They were left for 3 weeks in each desiccator and were weighed before each transfer. At the termination of the experiment the moisture content of each sample was determined and from this the moisture content at the end of each successive storage period was calculated. Seeds treated with petroleum jelly were washed in petrol ether preparatory to the moisture analysis. The results are presented in Table I.

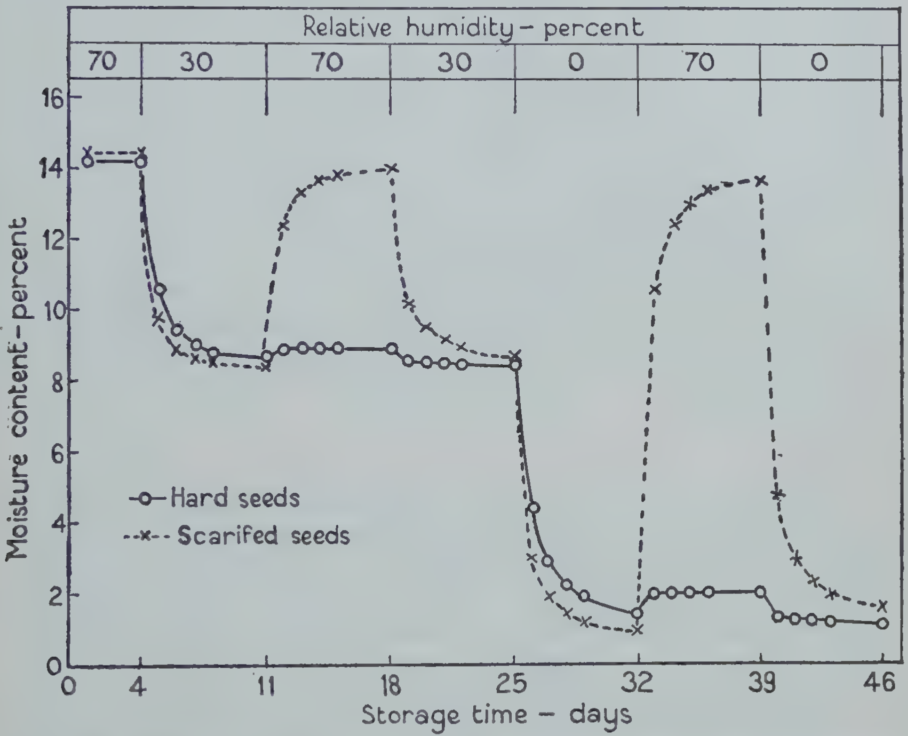

FIG. 4. Changes in moisture content occurring in white clover seeds transferred successively to chambers of different relative humidity.

TABLE I

Moisture Content (percentage of dry weight) of Seed Samples after Successive Periods of Storage under Different humidity Conditions

Relative Humidity. %	White clover		Red clover		
	Hard seeds.	Scarified seeds.	Hard seeds.	Hard seeds with hilum blocked.	Scarified seeds.
65	13·1	13·0	11·9	11·8	12·0
50	10·7	10·5	9·5	11·8	9·4
33	8·5	8·2	7·8	11·6	7·4
70	8·8	14·1	8·2	11·8	12·5
0	0·8	0·6	1·2	11·5	0·9
70	1·3	13·7	1·9	11·7	12·4

In scarified seed normal hygroscopic adjustment of the moisture content occurred. In the hard seeds desorption occurred as in the scarified seed but absorption was obstructed. Seeds with the hilum blocked neither gained nor lost water with changes in humidity. In these seeds desorption as well as absorption was obstructed. It is concluded that the testa of the hard seeds was impermeable except at the hilum. Water was lost from the hard seed through the hilum when the external relative humidity was low but it did not enter when the external humidity was high.

This is further confirmation of the conclusions reached in Experiment 1. An explanation of the results is found in the observations on the opening and closing of the hilar fissure. It appears that the hilum functions as a hygroscopic valve, opening in an atmosphere of low humidity and permitting additional drying of the seed, but closing in an atmosphere of high humidity with the result that the seed retains its dry condition.

The small increase in the weight of hard seeds observed in Experiment 1 to take place within the first day after a transfer to humid conditions may have been due to the absorption of water by tissues external to the impermeable layer, particularly the counter palisade and the spongy parenchyma at the surface of the hilum, and to the penetration of water vapour prior to the closing of the fissure.

Experiment 3

It was apparent from the previous experiments that a large and sudden increase in relative humidity brought about the closing of the hilar fissure and so prevented hard seeds from absorbing water and achieving hygroscopic equilibrium with the atmosphere. A further experiment was carried out to ascertain whether small increases in relative humidity had similar effects.

Four samples of approximately 1 g. were taken from a quantity of hard seeds of white clover ripened at 70 per cent. relative humidity. The samples were stored at 45 per cent. relative humidity for 21 days, during which time the moisture content fell from 14·1 to 10·0 per cent. Two samples were then transferred to a desiccator at 70 per cent. relative humidity, and two to a desiccator at 45 per cent. relative humidity equipped to permit the addition of water to the acid solution without removing the cover. Three millilitres of water were added daily through a tube leading to an inverted glass funnel suspended in the acid solution. By this means the density of the acid was gradually reduced over a period of 71 days from 1·350 to 1·243 and the relative humidity raised accordingly from 45 to 70 per cent. The samples were weighed periodically. Finally, one sample from each desiccator was analysed for moisture content and the other tested for germination. The results are shown in Figs. 5 and 6.

When the seed was subjected to a large and rapid increase in relative humidity absorption of water was obstructed, evidently by the closing of the hilar fissure. When the relative humidity was increased gradually absorption was not obstructed.

These results may be explained if it is assumed that to close the hilum there must be a difference in moisture tension exceeding a certain minimum between the palisade epidermis and the counter palisade which lie inside and outside the impermeable layer of the testa respectively. If, while the hilar fissure is open there is an increase in external relative humidity insufficient to produce this minimum difference the fissure remains open. Water vapour then diffuses into the seed until hygroscopic equilibrium is reached. It is presumably by the repetition of this process that the hard seeds were induced to absorb water under conditions of gradually increasing humidity.

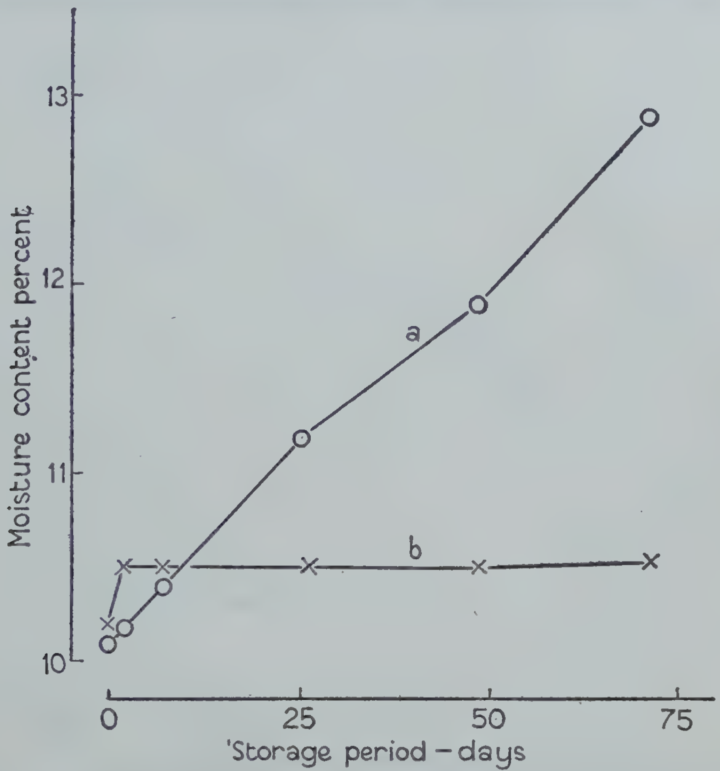

FIG. 5. Changes in the moisture content of hard seeds of white clover during storage. *a*, relative humidity increased gradually from 45 to 70 per cent.; *b*, relative humidity raised suddenly from 45 to 70 per cent. at the commencement of the storage period.

The germination tests indicate that the intensity of the hard-seed condition was greatly reduced by storage under conditions of gradually increasing humidity.

Observations on seed ripening

In the species studied the moisture content of seeds just prior to normal ripening ranged from 150 per cent. to 200 per cent. of the dry weight. Experiments were carried out to determine by what paths this water is lost during ripening, the speed at which it is lost, and the stage at which the testa becomes impermeable.

Experiment 4

From mature tree lupin pods which were just commencing to ripen three samples of six seeds were taken. The seeds in one sample were cut in two. In another the hilum of each seed was covered with petroleum jelly, while the third sample was untreated. Every seed was weighed separately and placed in a desiccator at 30 per cent. relative humidity. The seeds were reweighed periodically for 8 days. Seeds treated with petroleum jelly were finally washed in petrol-ether and then the moisture content of each seed was determined and its moisture content at each previous weighing calculated. Typical drying curves are shown in Fig. 7.

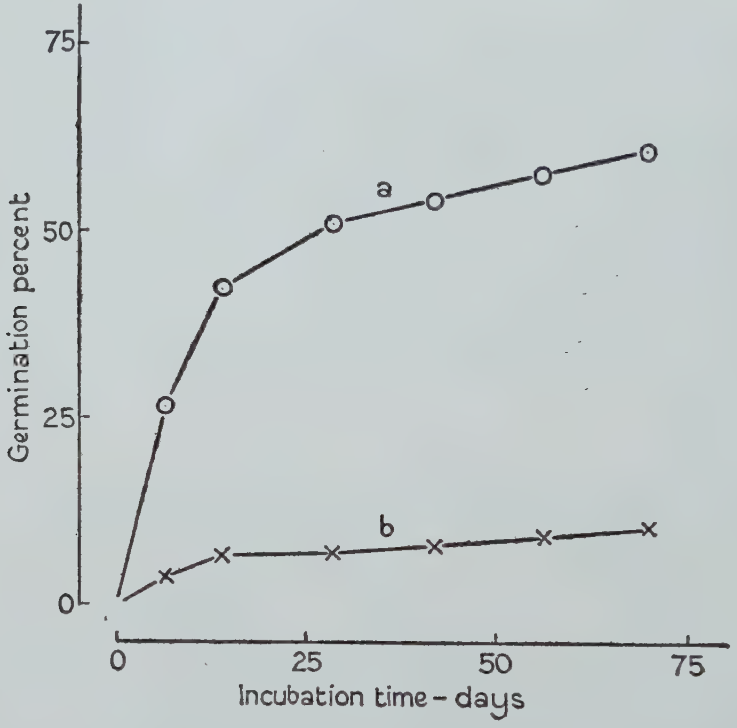

FIG. 6. Rate of germination of hard seeds of white clover previously stored under different conditions. *a*, seed stored under conditions of gradually increasing humidity; *b*, seed stored at constant high humidity.

The drying of the bisected seeds proceeded very rapidly and the equilibrium water content was reached within 24 hours.

In the ripening of untreated seed water was lost rapidly at first, but after 20 hours, when the moisture content had fallen to about 25 per cent., the rate of drying suddenly declined. Thereafter the moisture content continued to fall at a slow but steady rate until hygroscopic equilibrium level was reached on the sixth day.

During the first stage of ripening, seeds with the hilum blocked lost water at about the same rate as untreated seeds. There was similarly a reduction in rate of drying when the moisture content fell to about 25 per cent., but subsequent drying was at a lower rate than that of untreated seeds and it continued until the end of the experiment when the moisture content was 18 per cent. and still much above hygroscopic equilibrium level.

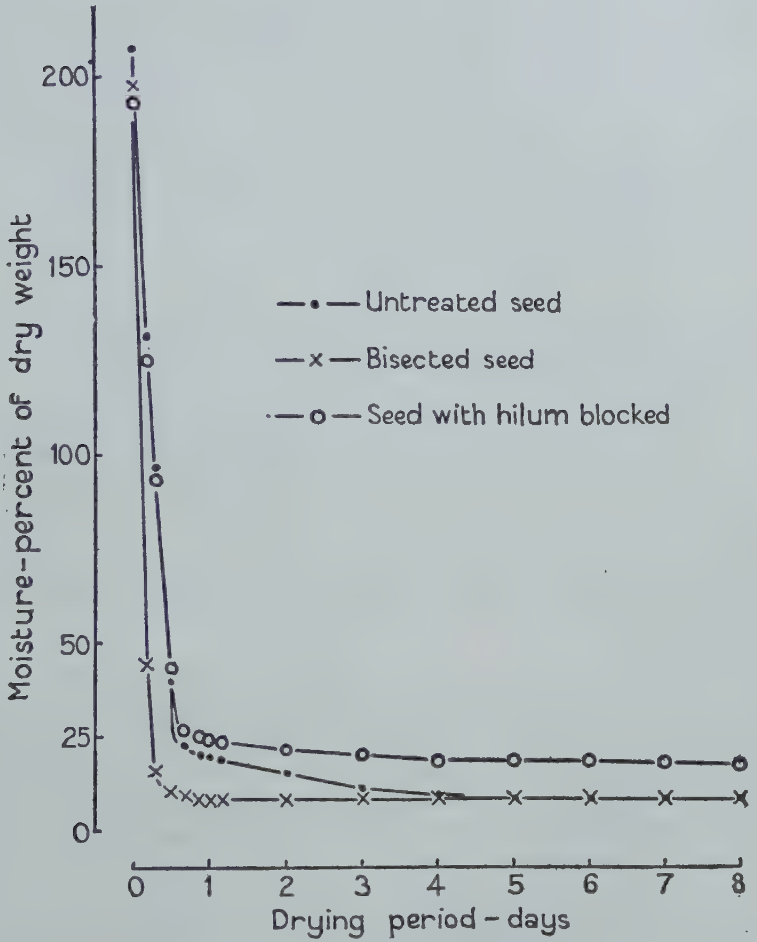

FIG. 7. Rate of drying of ripening seeds of tree lupin in a desiccator at 30 per cent. relative humidity.

It is apparent that after the moisture content of the untreated seeds had fallen to 25 per cent. most of the drying took place through the hilum.

Experiment 5

Seeds of tree lupin and red clover were collected when the pods had changed colours and were just beginning to shrink. Samples of 20 seeds of each species were taken and the hilum of each seed was covered with petroleum jelly. These samples were stored for 8 weeks in a desiccator at 35 per cent. relative humidity together with untreated samples of both species. The samples were weighed weekly. In the last week of storage there was no perceptible loss of weight in any sample. The samples treated with petroleum jelly were then washed in petrol-ether and finally all samples were analysed for water content. The results shown in Table II indicate when the moisture content falls to about 14 per cent. the epidermis becomes impermeable, so that no further loss of water from the seed can take place except through the hilum.

TABLE II

Moisture Content (percentage of dry weight) of Seeds Ripened at 35 per cent. Relative Humidity, showing the Effect of Blocking the Hilar Fissure

	Moisture Content	
Species.	Untreated seed.	Seed with hilum blocked.
Red clover	8·2	14·4
Tree lupin	9·3	14·9

Relation between moisture content of the seed and the duration of impermeability

Experiment 6

About 3,000 seeds of tree lupin were taken from pods which were commencing to ripen. They were spread thinly on a tray and stirred frequently. Periodically samples were drawn to determine the moisture content and also the median duration of impermeability. For the latter purpose 100 seeds were soaked in distilled water at 20° C. The imbibed seeds were counted periodically and the median duration of impermeability estimated by interpolation. The results are presented in Fig. 8.

While the moisture content exceeded 20 per cent. there was no perceptible delay in imbibition. As the moisture content fell below that level the time required for imbibition increased at an accelerating rate. There was not a sharp change from the permeable to the impermeable condition but below 14 per cent. moisture content the delay in imbibition increased very rapidly.

Experiment 7

Mature but unripe seed heads of white clover were collected and ripened at 5 different levels of relative humidity ranging from 30 to 70 per cent. The ripe seeds were rubbed out and soaked for 8 days at 20° C. The hard seeds were separated and dried quickly on filter paper. Each sample was then placed in a desiccator at the same relative humidity as that under which the seed was

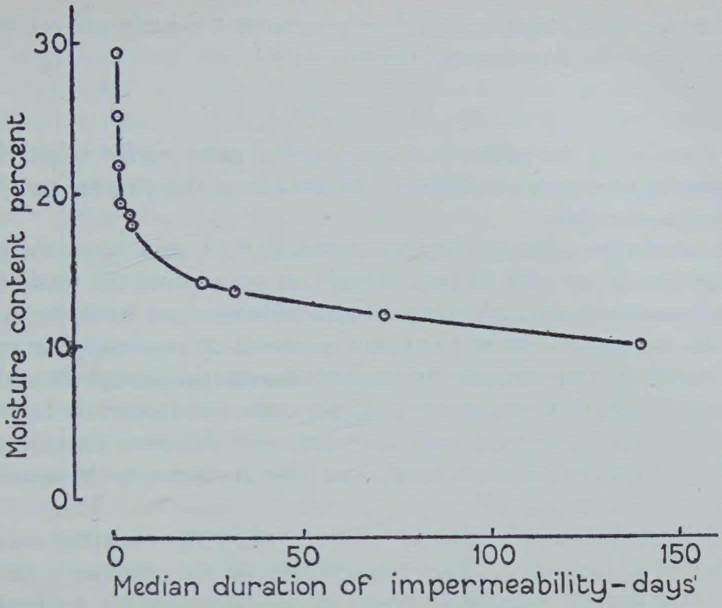

FIG. 8. Relationship between the moisture content of ripening seeds of tree lupin and the duration of the impermeable condition.

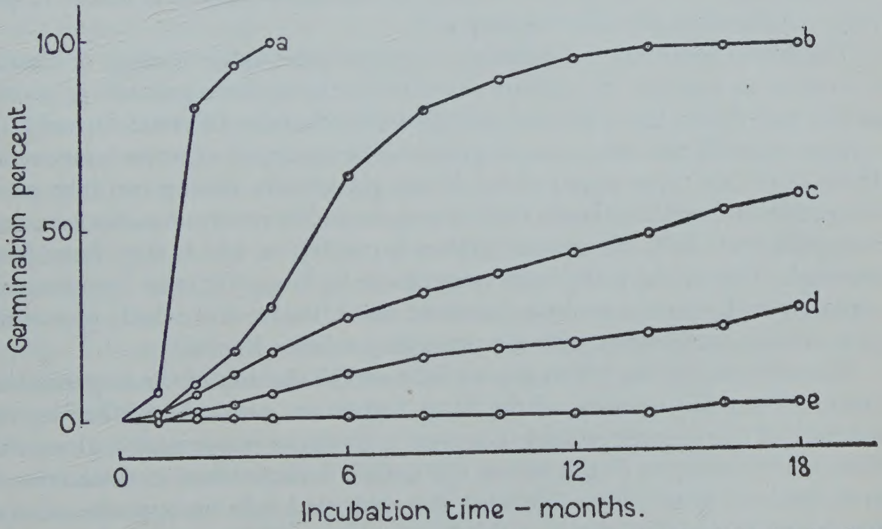

FIG. 9. Rate of germination of hard seeds of white clover ripened at the following levels of relative humidity: *a*, 70; *b*, 60; *c*, 50; *d*, 40; *e*, 30 per cent.

ripened. After 7 days the moisture content was determined and germination tests were commenced. The germination tests were carried out at 20° C. and were prolonged for 18 months. All seeds which germinated did so within 2 days of the first signs of imbibition. The results presented in Fig. 9 indicate that there is a high negative correlation between the moisture content of the hard seeds and the duration of impermeability.

DISCUSSION

The function of the hilum is comprehended most readily when the whole seed ripening process is considered. An outline of this process can be drawn from the present data.

As the seed ripens the moisture content falls from over 150 per cent. of the dry weight to 10 per cent. or less. Nearly 90 per cent. of the water lost from the seed passes out through the palisade epidermis and its covering cuticle. When the moisture content has fallen to about 25 per cent. the epidermis begins to resist the movement of water to the seed surface and when the moisture content falls to approximately 14 per cent. the epidermis becomes impermeable. The epidermis thus performs two different functions during ripening. At first it conducts water, but later it obstructs the movement of water.

The hilar mechanism begins to function when the moisture content has fallen below 25 per cent. The permeability of the epidermis then being restricted the counter palisade dries and shrinks rupturing the epidermis along the median groove of the hilum. Thereafter drying of the seed takes place increasingly by internal evaporation and the diffusion of water vapour outward through the hilar fissure. When the moisture content is below 14 per cent. drying takes place in this way only.

The hilum continues to function as a hygroscopic valve opening or closing according to whether the moisture tension in the counter-palisade is greater or less than that in the epidermal cells, provided that the difference in moisture tension exceeds the minimum required to bring about effective movement. Because of this valve action of the hilum progressive drying can take place intermittently, and hard seeds tend to acquire and to retain a moisture content in equilibrium with the lowest relative humidity to which they have been exposed. This explains the observation made by Guppy (5) that impermeable seeds generally have a moisture content much below that which represents hygroscopic equilibrium with the prevailing relative humidity.

The structure of the hilum throws light on the identity of the impermeable layer. When the margins of the hilar fissure are pressed together by the swelling of the counter palisade the testa is rendered impermeable along that line. At the margins of the fissure the cuticle is diminished in thickness. It is at the level of the 'light line' that the epidermal cells on opposite sides of the fissure first make contact as the fissure closes. This supports the conclusion of Coe and Martin (1) that the light line indicates the location of the impermeable layer. The fact that the compression of the surfaces of the fissure suffices to prevent the passage of water through the hilum suggests that impermeability throughout the epidermis is acquired in a similar way. In the upper part of each epidermal cell there are longitudinal thickening bands on the cell wall which occupy most of the cell cavity. As the seed shrinks during ripening the thickening bands become pressed together until they entirely obliterate the lumen of the cell and form an impervious layer. The further drying and shrinkage of the seed which is made possible by the hilar mechanism increases the compression of the palisade layer and intensifies its impermeability.

The observations on ripening seeds indicate the functions of some other tissues in the testa especially the hour-glass cells. During the early stages of ripening these cells conduct the water passing from the interior of the seed to the surface. As it ripens the seed shrinks and it is probably essential for the development of the impermeable condition that the epidermis should contract without excessive tangential stresses. Freedom from such stresses is ensured by the large intercellular spaces between the hour-glass cells and the restriction of thickening in these cells to longitudinal bands or in other species to an annular band about the middle of the cell. It is noteworthy that the epidermis has been shown by Hamly (6) to be most prone to split at the strophiole where it rests upon compact sclerenchyma. In the later stages of ripening the intercellular spaces between the hour-glass cells probably accelerate the drying of the seed by forming a path by which water vapour diffuses to the hilum. It is significant that the intercellular spaces between the hour-glass cells are continuous with those of the stellate cells surrounding the tracheid bar.

It was found that as the seeds ripened there was a continuous increase in the duration of impermeability. It is apparent therefore that the conventional definition of hard seeds is an arbitrary one which takes no account of the great variation in the intensity of the impermeable condition resulting from differences in the degree of desiccation.

The present study helps to reconcile some conflicting conclusions of previous workers regarding the hygroscopic behaviour of seeds of Leguminosae. Guppy (5) concluded that these seeds were non-hygroscopic because they did not change in weight with varying relative humidity. His seeds had a low moisture content and he did not store them in an atmosphere of sufficiently low relative humidity to cause opening of the hilum and additional drying. Ewart (4) observed that hard seeds lost weight during storage. It appears that unlike Guppy he was studying seeds under conditions of lower relative humidity than that in which they had previously been ripened. He rightly suspected that the seeds could give up water though incapable of absorption. Esdorn (3) observed seasonal fluctuations in the proportion of hard seeds in stored samples and concluded that this was due to normal hygroscopic responses to seasonal changes in relative humidity. In the present work it was found that under conditions of gradually increasing humidity hard seeds did absorb moisture and this suggests an explanation of Esdorn's observations.

CONCLUSIONS

In the papilionaceous seeds studied the hilum performs a function essential for the development of the hard seed condition. After the epidermis has become impermeable the hilum acts as a hygroscopic valve. It obstructs the entrance of water but permits the continued drying of the seed. This additional drying which the hilum makes possible intensifies the impermeability of the epidermis. The behaviour of the hilum explains how the development of an intensely impermeable testa can occur simultaneously with the desiccation of the interior of the seed.

ACKNOWLEDGEMENT

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